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### 4.17 Enzymes Involved in Processing Glutathione Conjugates

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| Section | Title | Page |
|---------|-------|------|
| 4.17.1 | Introduction | 325 |
| 4.17.2 | Enzymes of the Mercapturate Pathway | 326 |
| 4.17.2.1 | Glutathione S-Transferases | 326 |
| 4.17.2.2 | \(\gamma\)-Glutamyltransferase | 328 |
| 4.17.2.2.1 | GGT family of enzymes | 328 |
| 4.17.2.2.2 | Protein structure | 328 |
| 4.17.2.2.3 | Expression of GGT | 329 |
| 4.17.2.2.4 | Localization of GGT | 329 |
| 4.17.2.2.5 | Reactions catalyzed by GGT | 330 |
| 4.17.2.2.6 | Inhibitors of GGT | 330 |
| 4.17.2.2.7 | GGT and disease | 330 |
| 4.17.2.2.8 | \(\gamma\)-Glutamyl leukotriensase | 331 |
| 4.17.2.3 | Hydrolysis of Cysteinylglycine and its Conjugates | 331 |
| 4.17.2.3.1 | Aminopeptidase N | 331 |
| 4.17.2.3.2 | Cysteinylglycine dipeptidase | 332 |
| 4.17.2.3.3 | Leucyl aminopeptidase | 332 |
| 4.17.2.4 | Cysteine S-Conjugate N-Acetyltransferases | 332 |
| 4.17.3 | Interorgan Transport of Mercapturates and Mercapturate Intermediates | 333 |
| 4.17.4 | Biotransformations of Endogenous Compounds Through the Mercapturate Pathway | 336 |
| 4.17.4.1 | Deactivation/Detoxication | 336 |
| 4.17.4.2 | Bioactivation | 337 |
| 4.17.5 | Metabolic Pathways Diverging from Mercapturate Biosynthesis | 338 |
| 4.17.5.1 | Deconjugation | 338 |
| 4.17.5.2 | Deconjugation Coupled to Reductive Dehalogenation | 338 |
| 4.17.5.3 | Hydrolytic Deconjugation | 339 |
| 4.17.5.4 | Bis(glutathionyl) Conjugate Formation | 339 |
| 4.17.5.5 | Deamination and Transamination | 340 |
| 4.17.5.6 | Sulfur Oxidation | 340 |
| 4.17.5.7 | Thiomethyl Shunt | 341 |
| 4.17.5.8 | Aminoacylase-Catalyzed Deacylation of Mercapturic Acids | 341 |
| 4.17.6 | Cysteine S-Conjugate \(\beta\)-Lyases | 342 |
| 4.17.6.1 | Background – Bioactivation of Halogenated Alkenes | 342 |
| 4.17.6.2 | Possible Repair Mechanisms | 343 |
| 4.17.6.3 | The Cysteine S-Conjugate \(\beta\)-Lyase Reaction | 343 |
| 4.17.6.4 | Cysteine S-Conjugate \(\beta\)-Lyases in Enteric Bacteria | 344 |
| 4.17.6.5 | Cysteine S-Conjugate \(\beta\)-Lyases in Mammals | 345 |
| 4.17.6.6 | Cysteine S-Conjugate \(\beta\)-Lyases in Other Organisms | 345 |
| 4.17.6.7 | Reactive Sulfur-Containing Fragments Generated by \(\beta\)-Lyase Reactions Involving Halogenated Alkene Cysteine S-Conjugates | 347 |
| 4.17.6.8 | Catalytic Mechanism of the Cysteine S-Conjugate \(\beta\)-Lyase Reaction and Syncatalytic Inactivation | 348 |
| 4.17.7 | Major Cysteine S-Conjugate \(\beta\)-Lases of Mammalian Tissues | 350 |
| 4.17.7.1 | Glutamine Transaminase K/Kynurenine Aminotransferase I | 350 |
| 4.17.7.2 | Mitochondrial Aspartate Aminotransferase | 351 |
4.17.8 Role of the Mercapturate Pathway and Cysteine S-Conjugate β-Lyases in the Bioactivation of Toxic Halogenated Alkenes

4.17.9 Mechanisms Contributing to the Nephrotoxicity of Haloalkene Cysteine S-Conjugates – Toxicant Channeling

4.17.10 Toxic Homocysteine S-Conjugates

4.17.11 Electrophilic Xenobiotics Metabolized through the Mercapturate Pathway

4.17.11.1 General Considerations

4.17.11.2 Drugs Metabolized by the Mercapturate Pathway or by the Mercapturate/β-Lyase Pathway

4.17.12 Conclusions

References

Glossary

- **aminoacylase**: An enzyme that catalyzes the deacylation of N-acetyl-L-amino acids including mercapturates.
- **bioactivation**: A process by which a toxic compound is converted to a metabolite that is even more toxic.
- **S-conjugates**: The first conjugate to be formed in the mercapturate pathway is the glutathione S-conjugate. The reaction may occur spontaneously between glutathione and electrophile, but is most often catalyzed or accelerated by glutathione S-transferases. The glutathione S-conjugate is then successively converted to cysteinylglycine S-, cysteine S-, and N-acetyl cysteine S-conjugates.
- **cysteine S-conjugate β-lyases**: Pyridoxal 5’-phosphate (PLP)-containing enzymes that catalyze a net reaction in which a cysteine S-conjugate is converted to pyruvate, ammonium, and a sulfur-containing fragment.
- **cysteine S-conjugate N-acetyltransferase**: An enzyme that catalyzes the last step in the mercapturate pathway and that uses acetyl CoA as the acetyl donor.
- **deconjugation**: Removal of an intact cysteine-containing moiety (usually as glutathione) from the S-conjugate.
- **dipeptidases**: Enzymes that convert cysteinylglycine S-conjugates to cysteine S-conjugates and glycine, including aminopeptidase M, cysteinylglycine dipeptidase, and possibly leucyl aminopeptidase.
- **glutamine transaminase K (GTK)**: An enzyme of high specific activity in kidney cytosol, but present in most organs. The enzyme catalyzes reversible transamination with glutamine, aromatic amino acids, and a large number of sulfur-containing amino acids. It also catalyzes very effective cysteine S-conjugate β-lyase reactions. In addition, it is exceptionally active as both an aminotransferase and β-lyase with many selenocysteine Se-conjugates.
- **γ-glutamyl leukotrienease**: A unique enzyme of the γ-glutamyltransferase family that is highly selective for the glutathione S-conjugate of leukotriene (LTC₄), converting LTC₄ to LTD₄ (the cysteinylglycine S-conjugate of leukotriene).
- **γ-glutamyltransferase**: An enzyme that catalyzes the removal of the glutamate moiety from glutathione S-conjugates, generating the corresponding cysteinylglycine S-conjugates. Usually the acceptor molecule is water so that the other product of the reaction is glutamate. However, at exceptionally high concentrations, amino acids or dipeptides may be γ-glutamyl acceptors, generating a γ-glutamyl amino acid or a γ-glutamyl dipeptide, respectively.
- **glutathione S-transferases**: A family of enzymes that catalyzes the reaction of the cysteinyl anion of glutathione with an electrophile generating the corresponding glutathione S-conjugate.
- **mercapturate pathway**: A pathway that converts endogenous or exogenous electrophiles to their N-acetyl cysteine S-conjugate (mercapturate). The mercapturates are generally more polar and water soluble than the original electrophiles and are readily excreted in the urine or bile.
- **mitochondrial aspartate aminotransferase**: A very important enzyme in amino acid metabolism and present in high amounts in all tissues that contain mitochondria. The enzyme is important in linking amino acid metabolism to the TCA cycle (and to the urea cycle in the liver) and in the transfer of reducing equivalents from cytosol to mitochondrion in lieu of NADH. It is also a...
prominent mitochondrial cysteine S-conjugate β-lyase.

**thioacylation** A process whereby a reactive sulfur-containing fragment generated from a cysteine S-conjugate β-lyase reaction on a cysteine S-conjugate derived from a halogenated alkene reacts with ε-amino groups of key lysine residues in susceptible proteins.

**thiomethyl shunt** A series of reactions in which sulfur is added to an electrophile via the cysteine S-conjugate/C12-lyase pathway followed by an enzyme-catalyzed S-methylation reaction with S-adenosyl-L-methionine as methyl donor.

**toxicant channeling** A hypothesis in which it is suggested that reactive sulfur-containing species generated from toxic cysteine S-conjugates are channeled from the active site of mitochondrial aspartate aminotransferase to α-ketoglutarate dehydrogenase complex, thereby accounting for the remarkable sensitivity of this enzyme complex to inactivation in tissues/cells exposed to certain toxic cysteine S-conjugates.

### Abbreviations

- **AARE**: acylamino acid-releasing enzyme
- **AGAT II**: alanine-glyoxylate aminotransferase isoenzyme II
- **AlaAT**: alanine aminotransferase
- **ANIT**: 1-naphthylisothiocyanate
- **AOA**: aminooxyacetate
- **APN**: aminopeptidase N
- **AspAT**: aspartate aminotransferase
- **BCATc**: cytosolic branched-chain aminotransferase
- **BCATm**: mitochondrial branched-chain aminotransferase
- **BCKADHC**: branched-chain keto acid dehydrogenase complex
- **BTC**: S-(2-benzothiazolyl)-L-cysteine
- **CCBL1**: cysteine conjugate beta-lyase 1
- **Cyt**: cytosolic
- **DCVC**: S-(1,2-dichlorovinyl)-L-cysteine
- **DCVHC**: S-(1,2-dichlorovinyl)-L-homocysteine
- **FMO**: flavin-dependent monooxygenase
- **GGT**: γ-glutamyltransferase
- **GSH**: γ-glutamylcysteinylglycine
- **GST**: glutathione S-transferase
- **GTK**: glutamine transaminase K
- **GTL**: glutamine transaminase L
- **KAT I**: kynurenine aminotransferase isoenzyme I
- **KGDHC**: α-ketoglutarate dehydrogenase complex
- **KMB**: α-keto-γ-methylbutyrate
- **mit**: mitochondrial
- **PCBC**: S-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine
- **PD**: Parkinson disease
- **PDHC**: pyruvate dehydrogenase complex
- **PG**: prostaglandin
- **PLP**: pyridoxal 5’-phosphate
- **PMP**: pyridoxamine 5’-phosphate
- **rhGTK**: recombinant human GTK
- **TCA**: tricarboxylic acid
- **TFEC**: S-(1,2,2-tetrafluoroethyl)-L-cysteine
- **THT**: tetrahydrothiophene
- **THT-A**: β-(S-tetrahydrothiophenium)-L-alanine
- **UTR**: untranslated region

### 4.17.1 Introduction

Mercapturic acids (S-(N-acetyl)-L-cysteine conjugates) were first identified over 130 years ago and were soon recognized to play a role in the detoxication of certain halogenated xenobiotics (Bauman and Preusse 1879). However, it was not until 1959 that the tripeptide glutathione (γ-glutamylcysteinylglycine, GSH) was recognized as the source of the cysteiny1 portion of mercapturates (Barnes et al. 1959; Bray et al. 1959). GSH is found in almost all bacterial, plant, and mammalian cells in high concentration, typically 0.5–12 mM (Stevens and Jones 1989; Taniguchi et al. 1989). The mercapturate pathway begins with the
formation of a thioether bond between GSH and a xenobiotic in a reaction catalyzed (or enhanced) by glutathione S-transferases (GSTs). Subsequently, the resulting glutathione S-conjugate is converted to a cysteinylglycine S-conjugate by γ-glutamyltransferase (GGT) and thence to a cysteine \( S \)-conjugate by either aminopeptidase N (APN) or cysteine aminopeptidase (cysteinylglycinase) or possibly leucyl aminopeptidase. Finally, the cysteine \( S \)-conjugate or possibly leucyl aminopeptidase. Finally, the cysteine \( S \)-conjugate is converted to a cysteine \( S \)-conjugate by either aminopeptidase N (APN) or cysteine aminopeptidase (cysteinylglycinase) or possibly leucyl aminopeptidase. Finally, the cysteine \( S \)-conjugate is \( N \)-acyetylated to the corresponding \( N \)-acyetyl-L-cysteine \( S \)-conjugate (mercapturate). The mercapturate is generally more water soluble than is the parent compound and is more readily excreted. (For reviews, see Chasseaud 1976; Silbernagl and Heuner 1993; Stevens and Jones 1989.) Reactions involved in and associated with the mercapturate pathway are shown in Figure 1.

Reactions involved in drug metabolism were originally assigned to two general categories (Williams 1959). Phase I transformations serve to unmask a functional group and may involve reactions such as oxidation, hydrolysis, and reduction. Phase II transformations generate highly polar derivatives (conjugates), such as glucuronides and sulfate esters, which are excreted into the urine. Almost all these transformations are catalyzed by transferases (Cole and Deeley 2006). It was originally thought that phase II reactions complete the processes begun by phase I transformations. Others have suggested that elimination of the glutathione \( S \)-conjugate from the cell may represent a phase III detoxication event (Ishikawa 1992). It is now apparent that many xenobiotics are metabolized entirely by pathways that do not involve phase I reactions. Thus, many glutathione \( S \)-conjugates are formed from drugs and other xenobiotics without prior transformation. Moreover, glutathione \( S \)-conjugates are rarely excreted by the kidney because of their large size and amphiphilic character, and the presence of GGT and dipeptidases in the proximal tubules. Glutathione \( S \)-conjugates are often excreted into the bile (Dekant et al. 1994; Marchand et al. 1988; Silverman 1992) and/or further metabolized, especially to the mercapturate. As a result of ambiguities in classification, some authors have suggested that it may be time to discontinue the designations 'phase I' and 'phase II' metabolism (Josephy et al. 2005).

Notwithstanding how one classifies the transformations of xenobiotics through the mercapturate pathway, it is clear that the mercapturate pathway is a biochemical defense against potentially harmful electrophilic xenobiotics. Moreover, in special cases the pathway may serve also to metabolize endogenously generated substances such as leukotrienes (Sections 4.17.2.2.8 and 4.17.4.1). However, in some cases a diversion of the pathway leads to the bioactivation of xenobiotics rather than to a detoxication. Thus, cysteine \( S \)-conjugates formed from some halogenated alkenes are converted to pyruvate, ammonium, and a reactive, toxic fragment by the action of cysteine \( S \)-conjugate \( \beta \)-lyases (Section 4.17.6.1). Leukotriene transformations and cysteine \( S \)-conjugate \( \beta \)-lyases are considered in more detail below.

The purpose of this chapter is to provide a review of the enzymes involved in the mercapturate pathway and their biological importance in relation to reactions with endogenous and xenobiotic substances. The chapter also deals at some length with nonenzymatic and enzymatic processes that divert glutathione \( S \)- and cysteine \( S \)-conjugates away from the mercapturate pathway.

Some of the points discussed in this chapter have been adapted from recent reviews by Cooper and Pinto (2008) and by Anders (2008).

### 4.17.2 Enzymes of the Mercapturate Pathway

#### 4.17.2.1 Glutathione \( S \)-Transferases

These enzymes are described in detail in Chapter 4.16, so that only a few pertinent points will be discussed here. GSH has many biological roles, including (1) defense against reactive oxygen species and free radicals, (2) defense against reactive electrophiles, (3) carrier of cysteine in a less reactive form, (4) cofactor in several enzyme reactions, (5) participation in eicosanoid biology, (6) maintenance of cellular redox state, and (7) transport of cysteine (e.g., Taniguchi et al. 1989). Many drugs and other xenobiotics, or their metabolites, are directly electrophilic and are thus capable of causing cellular damage \emph{in vivo}. By intercepting these potentially harmful electrophiles, GSH is important in the defense against foreign substances.

GSH is a 'soft' nucleophile (i.e., the sulfur is of low electronegativity, high polarizability and is easily oxidized) and preferentially reacts with 'soft' electrophiles (i.e., compounds that contain an acceptor atom with high polarizability, low electronegativity, and often unshared \( p \) or \( d \) valence electrons). These reactions often proceed at a measurable rate in the absence of a catalyst (Koob and Dekant 1991), but in general the reaction is
Figure 1  The mercapturate pathway and associated side reactions. If the mercapturate precursor contains an electrophilic center it may react directly with GSH (reaction 1). Alternatively, the precursor may be converted to a compound with an electrophilic center (reaction 2) prior to reaction with GSH. Reactions 1 through 5 represent the mercapturate pathway. Reactions 7, 8, and 9 are alternative reactions for elimination of cysteine S-conjugate. Reactions 7 plus 8 denote the thiomethyl shunt. The thiomethyl compound (XSCH₃) may be excreted unchanged or further oxidized to sulfoxide, sulfone, or CO₂ and sulfate, which are excreted. For some cysteine S-conjugates metabolism may also involve conversion to the α-keto acid, α-hydroxy acid, and oxidatively decarboxylated product (see Section 4.17.5.5). The cysteine S-conjugate may also be converted to the corresponding sulfoxide. Enzymes involved: (1) glutathione S-transferases, (2) oxidases that generate an electrophilic center for attack by GSH (in some cases oxidation may be nonenzymatic), (3) ectoenzyme GGT, (4) ectoenzymes APN/cysteinylglycine dipeptidase and possibly cytosolic leucyl aminopeptidase, (5) N-acetyltransferases, (6) aminoacylases, (7) cysteine S-conjugate β-lyases, (8) thiomethyltransferase, (9) UDP-glucuronosyltransferases. In vivo the hydrolysis reaction of GGT predominates over the formation of γ-glutamyl amino acids. AdoHcy, S-adenosyl-L-homocysteine; AdoMet, S-adenosyl-L-methionine; A, amino acid, dipeptide, or GSH acceptor for the GGT reaction; γ-GLU-A, γ-glutamyl amino acid (or γ-glutamyl dipeptide; γ-glutamylglutathione). Many potentially toxic xenobiotics and a few endogenous compounds are metabolized through the mercapturate pathway. Silbernagl, S.; Heuner, A. In *Renal Disposition and Nephrotoxicity of Xenobiotics*; Anders, M. W., Dekant, W., Henschler, D., Oberleithner, H., Silbernagl, S., Eds.; Academic Press, Inc.: San Diego, CA, 1993; pp 135–154; Cooper, A. J. L.; Pinto, J. T. In *Biotechnology: Pharmaceutical Aspects. Advances in Bioactivation Research*; Elfarra, A. A., Ed.; Springer: New York, 2008.
accelerated by the action of GSTs. These enzymes are widespread in nature and are present in most mammalian tissues. In the mammalian liver, GSTs account for >3–10% of the soluble protein (Ketterer and Christodoulides 1994). The cytosolic forms are dimers with subunit \( M_s \) of \( \sim 25 \times 10^3 \). Many GST isoforms have been identified in the rat (Ketterer and Christodoulides 1994). Cytosolic GSTs are now usually classified into several classes (A (alpha), M (mu), P (pi), T (theta), Z (zeta), O (omega), and S (sigma)) according to their isoelectric point, substrate and inhibitor properties, antibody recognition, and N-terminal amino acid sequences. GSTs may also be broadly classified on the basis of their cellular location, namely microsomal, mitochondrial, and cytosolic (Hayes et al. 2005). The enzymes contain a binding site for GSH (nucleophile) and a separate binding site for hydrophobic compounds with electrophilic centers. GSTs are highly specific for GSH as nucleophile, but, depending on the isoform, exhibit wide and overlapping substrate specificities with regard to the electrophilic substrate. Binding of GSH to the active site facilitates ionization to the nucleophilic thiolate anion \( \text{GS}^- \) (Ketterer and Christodoulides 1994).

GSH conjugation can occur by three mechanisms (Anders 2004, 2008). For example, haloalkenes and dichloroacetylene undergo GST-catalyzed vinylic substitution (SNV) reactions that may be either a Michael addition reaction (e.g., with tetrafluoroethylene (eqn [1]), dichloroacetylene (eqn [2]) or an addition elimination reaction (e.g., with trichloroethylene (eqn [3])). GSTs also catalyze reactions with epoxide moieties (e.g., in the conversion of leukotriene \( \text{A}_4 \) (LT\( \text{A}_4 \)) to leukotriene \( \text{C}_4 \) (LT\( \text{C}_4 \))). Glutathione \( S \)-conjugate formation with haloalkenes is catalyzed by both microsomal (MGST1) and cytosolic GSTs in ratios that depend to some extent on the structure of the haloalkene (Cooper and Pinto 2008, and references cited therein).

\[
\begin{align*}
\text{F}_2\text{C}=&\text{CF}_2 + \text{GS}^- + \text{H}^+ \rightarrow \text{F}_2\text{C}(\text{H})\text{CF}_2\text{SG} \quad (1) \\
\text{ClC}=&\text{CCl} + \text{GS}^- + \text{H}^+ \rightarrow \text{ClC(\text{H})=C(\text{Cl})SG} \quad (2) \\
\text{Cl(\text{H})=C}=\text{CCl} + \text{GS}^- \rightarrow \text{ClC(\text{H})=C(\text{Cl})SG} + \text{Cl}^- \quad (3)
\end{align*}
\]

Interestingly, despite their generally broad specificity, many GSTs are able to catalyze the stereoselective addition of GSH to some electrophiles generating predominantly one enantiomer (Dohn et al. 1985; Livesey et al. 1982; Mangold and Abdel-Monem 1980).

### 4.17.2.2 \( \gamma \)-Glutamyltransferase

#### 4.17.2.2.1 GGT family of enzymes

In 1959, it was postulated that a glutathionase (an enzyme that degrades GSH) played a role in the formation of mercapturic acids (Bray et al. 1959). Previously, Binkley and Nakamura had demonstrated that the first step in the breakdown of GSH is cleavage of the \( \gamma \)-glutamyl bond followed by cleavage of the cysteinylglycine peptide bond (Binkley and Nakamura 1948). The enzyme that cleaves the \( \gamma \)-glutamyl bond of GSH and glutathione \( S \)-conjugates was subsequently isolated and named \( \gamma \)-glutamyltransferase (GGT; also known as \( \gamma \)-glutamyltranspeptidase) (Orlowski and Meister 1965; Szewczuk and Baranowski 1963). A second enzyme that cleaves \( \gamma \)-glutamyl bonds (converting the glutathione \( S \)-conjugate LT\( \text{C}_4 \) to the corresponding cysteinyl-glycine conjugate LT\( \text{D}_4 \)) was identified independently by two groups in the 1990s and named GGT-rel and \( \gamma \)-glutamyl leukotrienate (Carter et al. 1997; Heisterkamp et al. 1991). In humans, the genes that encode GGT and \( \gamma \)-glutamyl leukotrienate are located in close proximity on the long arm of chromosome 22, in an area of the genome that has undergone multiple duplications (Bailey et al. 2002; Bulle et al. 1987; Heisterkamp et al. 1991). With the cloning of the human genome, GGT pseudogenes and genes encoding truncated portions of GGT have been identified, in addition to several genes that theoretically could encode a full-length protein (Heisterkamp et al. 2008). To date only GGT and \( \gamma \)-glutamyl leukotrienate have been shown to be expressed as functional proteins. Heisterkamp and colleagues recently collaborated with the HUGO (Human Genome Organization) Gene Nomenclature Committee to standardize the nomenclature for the GGT gene family (Heisterkamp et al. 2008). The new nomenclature designates \( \gamma \)-glutamyltransferase as GGT1 and \( \gamma \)-glutamyl leukotrienate as GGT5. For clarity throughout this chapter, we will continue to use the descriptive names \( \gamma \)-glutamyltransferase (GGT) and \( \gamma \)-glutamyl leukotrienate. Homologues of GGT are expressed throughout the plant and animal kingdoms (Martin et al. 2007; Morrow et al. 2007; Ubiyvovk et al. 2006).

#### 4.17.2.2.2 Protein structure

GGT is a type II protein with a single transmembrane domain within the large subunit (Coloma and Pitot 1986; Goodspeed et al. 1989; Rajpert-De Meyts et al. 1988; Sakamura et al. 1988). Human
GGT consists of 569 amino acids (Goodspeed et al. 1989; Rajpert-De Meyts et al. 1988; Sakamuro et al. 1988). Only the first four amino acids of the N-terminus extend into the cytosol (see Figure 2). All of the enzymatic activity takes place in the extracellular portion of the enzyme (Horiuchi et al. 1978; Tsao and Curthoys 1980). The enzyme is translated as a single polypeptide chain (Nash and Tate 1982). In the endoplasmic reticulum, the protein is cleaved into two subunits that remain noncovalently bound (Kinlough et al. 2005; Nash and Tate 1982). Both subunits are required for activity (Gardell and Tate 1981). The enzyme contains seven potential N-glycan sites. N-Linked glycans have been isolated from GGT and characterized (Yamashita et al. 1983, 1986). GGT in rat has been shown to contain two O-linked glycans, but no O-linked glycans have been reported for human GGT (Blochberger et al. 1989). GGT in rat has been shown to contain two O-linked glycans, but no O-linked glycans have been reported for human GGT (Blochberger et al. 1989). Cleavage of the large subunit by papain or bromelain releases a hydrophilic form of the enzyme that is catalytically indistinguishable from the intact membrane-bound enzyme (Ikeda et al. 1995b). The crystal structures of GGT homologues from Escherichia coli and Helicobacter pylori have been reported, but mammalian GGT has not yet been crystallized (Morrow et al. 2007; Okada et al. 2006).

**Figure 2** Diagrammatic structure of γ-glutamyltransferase (GGT). The enzyme is composed of two noncovalently attached subunits. The larger subunit traverses the cellular membrane such that most of the protein is exposed to the extracellular milieu. The smaller subunit is entirely within the extracellular milieu. As a result of its location, GGT is often referred to as an ectoenzyme. GGT may catalyze the hydrolysis of the γ-glutamyl compound to free glutamate and R or catalyze reactions with an acceptor (transpeptidation) to generate γ-glutamyl-acceptor and R. The acceptor may be an amino acid, dipeptide, or GSH. Under physiological conditions hydrolysis predominates over transferase reactions.

4.17.2.2.3 Expression of GGT

Five distinct mRNAs that encode GGT have been identified in humans, six in mice, and multiple mRNAs have also been identified in rats and pigs (Chikhi et al. 1999; Visvikis et al. 2001). In each species, the mRNAs all encode the same protein but differ in their 5’-untranslated region (UTR). The sequence of the UTR reveals use of multiple promoters that are regulated in a tissue-specific manner. In the mouse, alternative splicing of the mRNA has been shown to produce at least three GGT-isoforms (Joyce-Brady et al. 2001). One of the splicing events is developmentally regulated (Joyce-Brady et al. 2001).

4.17.2.2.4 Localization of GGT

GGT is expressed on the apical surface of glands and ducts of many tissues within the human body (Hanigan and Frierson 1996). The highest level of GGT expression is on the surface of the renal proximal tubules. Secretory and absorptive epithelial cells in glands in the testes, endocervix, endometrium, adrenals, prostate, intestinal crypts, sweat glands, and salivary glands all express GGT. In the liver, GGT is localized to the bile canaliculi of hepatocytes and bile ducts. GGT is also expressed on the apical surface of pancreatic acinar
cells, and activity can be detected in pancreatic juice and bile (Kristiansen et al. 2004). Capillary endothelium in the brain and spinal cord also express GGT. GSH and glutathione S-conjugates present in fluids that flow through these ducts are metabolized by GGT.

4.17.2.2.5 Reactions catalyzed by GGT
GGT catalyzes the cleavage of \( \gamma \)-glutamyl bonds (Figure 2). The \( \gamma \)-glutamyl group of the substrate is transiently bound to the enzyme and the reaction proceeds through a modified ping-pong mechanism (Keillor et al. 2005). In the human enzyme, arginine 107 and aspartate 423 are implicated in binding the \( \alpha \)-carboxylate and \( \alpha \)-amino group, respectively, of the \( \gamma \)-glutamyl moiety (Ikeda et al. 1995a). Cleavage of the \( \gamma \)-glutamyl bond releases the glutamyl donor as the first reaction product. The \( \gamma \)-glutamyl group is released from the enzyme when the enzyme-\( \gamma \)-glutamyl complex reacts with either a water molecule, yielding glutamate, or with the \( \alpha \)-amine of an amino acid (transferase or transpeptidase reaction), yielding a \( \gamma \)-glutamyl amino acid (Taniguchi and Ikeda 1998). The \( \alpha \)-amine of a free amino acid or a dipeptide can serve as an acceptor for the \( \gamma \)-glutamyl group (Thompson and Meister 1977). The enzyme is routinely assayed by the development of a yellow color upon the release of \( p \)-nitroanaline from \( \gamma \)-glutamyl-\( p \)-nitroanilide. The addition of an acceptor such as glycylglycine in a large excess (10- to 40-fold excess relative to the substrate concentration) accelerates the rate of the reaction. The standard biochemical assay is conducted at pH 8.5, the pH optimum of the transferase reaction.

Analysis of the kinetics of the reaction indicated that, in the kidney, the hydrolysis reaction predominates under physiological conditions due to the reduced pH in the proximal tubule and the rapid removal of amino acids (Curthoys and Hughey 1979). The essential nature of this reaction was established with the development of GGT knockout strains of mouse (Lieberman et al. 1996). GGT-null mice were born at expected ratios with no apparent phenotypic abnormalities. However, they excreted high levels of GSH in their urine, failed to grow at the same rate as their wild-type littermates, and died by 10 weeks of age from a cysteine deficiency. GGT-null mice can be rescued by supplementing them with N-acetyl cysteine. Earlier work with cultured cell lines had shown that expression of GGT enabled cells to initiate the release of cysteine from extracellular GSH (Hanigan and Ricketts 1993). GSH cannot be taken up intact by most cells. In the knockout mice, GSH present in the glomerular filtrate passes intact through the proximal tubules and is excreted into the urine (Lieberman et al. 1996). In wild-type mice, GGT localized to the apical surface of the proximal tubules cleaves the \( \gamma \)-glutamyl bond releasing glutamate and cysteinylglycine. The cysteinylglycine dipeptide can be cleaved into cysteine and glycine by any of several dipeptidases present on the apical surface of the proximal tubule cells (Section 4.17.2.3). Cysteine can be synthesized from methionine or through a salvage pathway, but the knockout mice are not able to maintain an adequate supply of cysteine for growth and development while continuously excreting large amounts of GSH in the urine. The GGT knockout mouse becomes deficient only in cysteine, indicating that uptake of amino acids does not depend on GGT, as had been proposed by some investigators (Orlowski and Meister 1970). A physiological role for the transferase reaction has not been demonstrated.

4.17.2.2.6 Inhibitors of GGT
Glutamine analogues including acivicin, 6-diazo-5-oxo-L-norleucine, and \( l \)-azaserine inhibit GGT (Taniguchi and Ikeda 1998). \( \gamma \)-Phosphono diester analogues of glutamate have also been shown to be potent inhibitors of GGT (Han et al. 2007). \( l \)-Alkyl \( l \)-homocysteine analogues function as competitive inhibitors (London and Gabel 2001). Serine-borate and analogues of the serine-borurate complex are transition-state inhibitors (London and Gabel 2001; Tate and Meister 1978).

4.17.2.2.7 GGT and disease
The half-life of GGT in rat renal tissue is 35 h (Capraro and Hughey 1985). The enzyme is induced by oxidative stress through the ras signal transduction pathway (Pandur et al. 2007). GGT is expressed by many tumors and contributes to resistance to chemotherapy (Hanigan et al. 1999a,b; Pomppella et al. 2006). GGT is upregulated in preneoplastic foci of hepatocytes in rats treated with toxic hepatocarcinogens (Hanigan and Pitot 1985). Elevated levels of enzymatically active GGT can be detected in the serum of patients with liver and pancreatic disease, including inflammatory disease and cancer.
(Engelken et al. 2003; Whitfield 2001). GGT activity is also detectable in some commercial lots of bovine serum that are used to maintain cells in culture and can affect studies involving GSH synthesis and regulation (Hanigan et al. 1993).

4.17.2.2.8 γ-Glutamyl leukotrienase

In 1991, Heisterkamp and coworkers identified a GGT related gene, GGT-rel, in a human cDNA library (Heisterkamp et al. 1991). The new gene named GGT-rel cleaved the γ-glutamyl bond of LTC₄ (a glutathione S-conjugate) but did not cleave the γ-glutamyl substrates routinely used to assay GGT activity. The nucleotide sequence revealed 39.5% identity between the amino acid sequences of GGT and GGT-rel. The similarity included a hydrophobic domain (amino acids 12–28) similar in location to the transmembrane domain of GGT, indicating that GGT-rel is also a type II membrane protein with the active site on the cell surface. The ability of the GGT-null mouse to cleave the γ-glutamyl bond of LTC₄ and release LTD₄ led Carter and coworkers to initiate a search for another enzyme that could cleave γ-glutamyl bonds. They identified an enzyme in the mouse they called γ-glutamyl leukotrienease, which has since been shown to be the mouse homologue of GGT-rel (Carter et al. 1997). There is also a GGT-rel homologue in the rat (Potdar et al. 1997). Heisterkamp and colleagues presented data that ‘suggested’ GGT-rel could cleave the γ-glutamyl bond of GSH. However, tissues from the GGT-null mouse, which expresses GGT-rel, did not catalyze any detectable cleavage of GSH or glutathione disulfide (GSSG) indicating that GGT-rel has very weak, if any affinity for GSH as a substrate (Carter et al. 1997; Heisterkamp et al. 1991). Acivicin inhibits both GGT-rel and GGT, but glycylglycine does not accelerate the velocity of GGT-rel as is observed for GGT (Carter et al. 1994, 1997).

GGT-rel is expressed by endothelial cells and is localized to the capillaries and sinusoid throughout the body (Carter et al. 1994). Comparison of kidney homogenates from wild-type and GGT-null mice for their ability to convert LTC₄ to LTD₄ demonstrates that GGT can cleave LTC₄. However, studies with GGT-null and GGT-rel null mice indicate that in vivo GGT does not metabolize LTC₄ to LTD₄, which may be due to the localization of GGT in ducts and glands, sites distinct from those that synthesize LTD₄ (Carter et al. 1994; Han et al. 2002).

4.17.2.3 Hydrolysis of Cysteinylglycine and its Conjugates

4.17.2.3.1 Aminopeptidase N

The sequence of human (APN synonyms: aminopeptidase M, peptidase E, peptidase I, alanine dipeptidase, alanyl aminopeptidase) was deduced from the cDNA (Olsen et al. 1988). The cDNA encodes 967 amino acids per subunit and the sequence contains 11 possible glycosylation sites. Over the last 20 years many related enzymes have been sequenced. APN is a type II metalloproteinase that belongs to the M1 family (Luan and Xu 2007). The M1 family consists of at least nine members, five of which are integral membrane proteins (Albiston et al. 2004). APN is important in modulating the activity of several peptide hormones (Albiston et al. 2004; Luan and Xu 2007). The enzyme also serves as a receptor for coronaviruses and other human viruses, and influences immune function, cell proliferation, secretion, and angiogenesis (Luan and Xu 2007).

APN, like GGT, is found in microvillus membranes in many tissues, but especially in renal proximal tubules, small intestine, and epididymis (Kozak and Tate 1982; Tate 1985, 1989). APN is a homodimer (M₀ of the subunit, 130 × 10⁶) that contains two zinc ions per enzyme dimer. The enzyme has a broad specificity toward dipeptides and oligopeptides, amides, and arylamides. Usually the N-terminal amino acid is cleaved. The best substrates generally contain an alanine residue at the N-terminus but almost any amino acid can substitute, including proline. When the N-terminus is a proline followed by a hydrophobic residue a dipeptide may be cleaved from the N-terminus (Tate 1985, 1989). As a result the enzyme can degrade a large number of peptides down to single peptides (or occasionally dipeptides). The enzyme hydrolyzes a number of endogenous peptides, including the thrombin receptor activation peptide (Coller et al. 1993). The human enzyme is identical to myeloid marker protein CD 13 (Look et al. 1989).

APN is anchored to the apical membranes of the kidney proximal tubules by a hydrophobic stretch of amino acids at the N-terminus. The enzyme, as is the case with GGT, can be readily released from membranes by treatment with papain. The amino acid sequence indicates that this enzyme, like GGT, is synthesized with an uncleaved signal sequence at its N-terminus, which serves as a membrane anchor (Tate 1989). The human enzyme has been shown to possess an additional sorting signal that allows the enzyme to be segregated in the trans-Golgi network.
and then to the apical membrane (Wessels et al. 1990). APN, like GGT, is glycosylated (Tate 1989). An intimate relationship exists between high mannose glycosylation and polypeptide folding during the synthesis of the mature enzyme, the timing of which is essential for correct polypeptide folding (Danielsen 1992).

4.17.2.3.2 Cysteinylglycine dipeptidase
Cysteinylglycine dipeptidase (P1644; synonyms: dipeptidase 1, cysteinylglycinase, microsomal dipeptidase, renal dipeptidase, dehydropeptidase 1) is present in the microvilli of epithelial cells. The enzyme is a dimer ($M_r$ of the monomer $\sim 50 \times 10^3$) and contains four zinc ions per enzyme dimer (Kozak and Tate 1982; Tate 1985, 1989). The rat enzyme is glycosylated, but the human kidney enzyme apparently is not (Campbell et al. 1984). The enzyme is selectively inhibited by cilastatin, whereas APN is selectively inhibited by bestatin. This selectivity has allowed Tate and coworkers to estimate that about 65, 60, and 95% of S-methyl-cysteinylglycine hydrolyzing activities of the rat renal, jejunal, and epididymal membranes are due to cysteinylglycine dipeptidase (reviewed in Tate 1989). Furthermore, cysteinylglycine dipeptidase is the major activity responsible for the hydrolysis of cystinyl-bis(glycine) and LTD$_4$ in these membranes (Kozak and Tate 1982; Tate 1989). The enzyme is also thought to be responsible for the hydrolysis of some $\beta$-lactam antibiotics such as perem and carperem (Campbell et al. 1984). The primary structure of the human cysteinylglycine dipeptidase has been deduced from the cDNA by Adachi et al. (1990b). The enzyme is located on the surface of the brush-border membranes (Tate 1989). The cDNA codes for 411 amino acid residues beginning with a signal peptide of 16 residues (Adachi et al. 1990a). A highly hydrophobic region of 16 amino acids was dedicated to reside at the C-terminus, but the sequence was later shown to be absent from the mature protein expressed in COS cells (Adachi et al. 1990a). The enzyme, unlike APN, is refractory to release from membranes with papain (Tate 1989). It is released, however, by phosphatidylinositol-specific phospholipase C (PI-PLC) (Tate 1989). The enzyme is converted from an amphipathic to a hydrophilic form by this treatment. Adachi et al. (1990a) showed that the enzyme is anchored to the membranes at a serine residue via glycosyl phosphatidylinositol.

Cysteinylglycine dipeptidase is more active with dipeptides such as cysteinylglycine, $\beta$-cystinyl-bis-(glycine) and S-methyl-$L$-cysteinylglycine than is APN (Kozak and Tate 1982).

4.17.2.3.3 Leucyl aminopeptidase
It is generally thought that APN and especially cysteinylglycine dipeptidase are the most important enzymes involved in the hydrolysis of cysteinylglycine and cysteinylglycine $S$-conjugates. However, Jösch et al. (1998) have reported that a cytosolic liver cysteinylglycinase catalyzes the hydrolysis of cysteinylglycine and model cysteinylglycine $S$-conjugates. Mammalian cells contain plasma membrane transporters for dipeptides and tripeptides (e.g., Rubio-Aliaga and Daniel 2002). Thus, formation of the cysteine $S$-conjugate may occur intracellularly as well as extracellularly. The cytosolic activity may be especially important in species such as guinea pig and human that exhibit high hepatic GGT activity (Jösch et al. 1998). The same group later identified the enzyme as manganese-dependent leucyl aminopeptidase (synonyms: cytosolic aminopeptidase, leucine aminopeptidase, leucine aminopeptidase 3, proline aminopeptidase, peptidase S; LAP3, LAPEP, PEPS) (Jösch et al. 2003). This enzyme also appears to be important in GSH turnover in bovine lens (Cappiello et al. 2004).

4.17.2.4 Cysteine S-Conjugate N-Acetyltransferases
The last step of the mercapturate pathway is catalyzed by $N$-acetyltransferases. A number of mostly cytosolic $N$-acetyltransferases are known and have been well characterized. These enzymes are discussed in detail in Chapter 4.19. Therefore, only salient points pertaining to the $N$-acylation of cysteine $S$-conjugates are discussed here. Acetyltransferase activity toward alkyl cysteine $S$-conjugates was detected in rat liver slices by Barnsley et al. (1969). Subsequently, an acetyltransferase that $N$-acylates cysteine $S$-conjugates (eqn [4]) was shown to be present in liver and kidney microsomes (Green and Elce 1975). Evidence has been presented that the activity in microsomes is due to a single enzyme (Birner et al. 1997). The enzyme exhibits some activity toward cysteine and methionine, but is much more active with $S$-benzyl-$L$-cysteine and other cysteine $S$-conjugates. It has no activity with glutamate or aspartate.
Cysteine S-conjugate N-acetyltransferase specific activity is highest in the kidney (Duffel and Jakoby 1982, 1985). Within the kidney, activity is highest in the straight portion of the proximal tubule (Heuner et al. 1991). The enzyme is located on the cytosolic surface of the endoplasmic reticulum (Okajima et al. 1984). The enzyme is difficult to remove from microsomes, but it has been successfully isolated and partially purified from pig kidney (Aigner et al. 1984). The purified enzyme was shown to be active with seven nephrotoxic cysteine S-conjugates derived from halogenated alkenes (Kraus et al. 2000). The purified enzyme was shown to be active with seven nephrotoxic cysteine S-conjugates derived from halogenated alkenes (Kraus et al. 2000). The $K_m$ values were remarkably similar (≈0.1–0.3 mM), but $V_{\text{max}}$ values were more varied.

### 4.17.3 Interorgan Transport of Mercapturates and Mercapturate Intermediates

Formation of glutathione S-conjugates in the body generally occurs mostly in the liver (Dekant et al. 1994). Cysteine S-conjugates and mercapturates that are formed in the liver are excreted by the kidneys and therefore their metabolism involves interorgan transport. The following general discussion applies mainly to the rat. However, as noted above, other mammals, such as guinea pigs and humans, have much higher levels of hepatic GGT (Hinchman and Ballatori 1990; Hinchman et al. 1991, 1993), and therefore metabolic pathways and trafficking of mercapturate intermediates may vary from species to species. We begin with an overview of inter-organ transport followed by a discussion of specific glutathione S-conjugate-transport proteins.

Although some transport of glutathione S-conjugates may occur across the sinusoidal membranes (e.g., Inoue et al. 1984a,b), the major route for the removal of these compounds in rat liver is via canicular transporters into the bile (e.g., Akerboom et al. 1991; Hinchman et al. 1998; Keppeler and König 2000; Vore 1993). The importance of the biliary excretion system is underscored by experiments in which 1-naphthylisothiocyanate (ANIT) is administered to experimental animals. ANIT is a hepatotoxicant that induces portal edema, parenchymal necrosis, and inflammation of the bile duct epithelium. ANIT reacts reversibly with GSH. The conjugate is released to the bile where the reverse reaction results in bile duct damage and increased GSH concentration (Jean et al. 1995). A number of compounds, including isothiocyanates and isocyanates (e.g., methylisocyanate, the compound responsible for the Bhopal tragedy), can react reversibly with GSH and thereby be disposed throughout the body (Bailie and Kassahun 1994). Biliary cannulation protects rats from the nephrotoxic effects of hexachloro-1,3-butadiene and $\beta$-aminophenol (Gartland et al. 1990; Nash et al. 1984). This finding also emphasizes the importance of the biliary excretion as a first step in complex multiorgan pathways involved in glutathione S- and cysteine S-conjugate metabolism (Dekant et al. 1994).

After secretion into the bile, glutathione S-conjugates may arrive intact at the small intestine or they may be sequentially degraded to the corresponding $\gamma$-glutamylcysteine and cysteine S-conjugates by the action of GGT and dipeptidases, respectively (Larsen and Bakke 1981). In this context, it is notable that although the specific activity of GGT in the whole adult rat liver is low, the specific activity locally in the luminal membranes of the bile duct epithelium and bile canalicular membranes of hepatocytes is much higher (Tate and Meister 1985). The mixture of S-conjugates in the gut may have at least four fates: (1) excretion with the feces; (2) absorption from the gut to the general circulation and eventual processing in the kidney; (3) recirculation through the enterohepatic system; and (4) metabolism within the gut lumen (Dekant et al. 1988, 1994). Catabolic reactions within the gut lumen may include conversion of cysteine S-conjugates to pyruvate, ammonium, and a thiol-containing fragment by bacterial cysteine S-conjugate $\beta$-lyases. The thiol metabolite may be converted to the thiomethyl derivative by thiol S-methyl transferase. The thiomethyl compound may be excreted or enter the circulation where it may be further oxidized to a sulfoxide and to a sulfone (Larsen and Bakke 1979). These reactions are considered in more detail in Sections 4.17.5.6 and 4.17.5.7. Some cysteine S-conjugates formed in the bile may reenter liver cells to be converted therein to the corresponding mercapturate.

Glutathione S- and cysteine S-conjugates are taken up by the gut, but the uptake of cysteine S-conjugates is generally more efficient (Gietl et al. 1991). The presence of GGT and dipeptidase in the microvilli of the brush border of the enterocytes (Tate and Meister 1985) ensures that at least some
of the glutathione S-conjugate is metabolized within the enterocyte, resulting in release of the cysteine
S-conjugate to the circulation. In some cases, the mercapturate may be formed in the small intestinal
cells and released to the circulation (Grafoström et al. 1979). However, in other cases, some glutathione
S-conjugate may enter the circulation from the brush-border membranes of the gut cells via a
mechanism that does not require GGT. This transporter may be identical with the GSH transporter
(Dekant et al. 1994).

GSTs are widespread among different tissues (Chapter 4.16). Therefore, it is possible that although glutathione S-conjugates are usually made preferentially in the liver, some may be made elsewhere in the body. Indeed, for several xenobiotics a major site for
cysteine S-conjugate formation appears to be the kidney (Hassal et al. 1984; Kanhai et al. 1989; Koob and
Dekant 1990). The glutathione S-conjugates released to the circulation from the liver, gut, and other organs or generated directly in the kidney may be further processed in the kidney. The
kidneys efficiently remove GSH and glutathione S-conjugates from the circulation by filtration (~25%) and by basolateral uptake (~75%) (Schrenk et al. 1988). Glutathione
S-conjugates transported in the blood are generally too small to be retained by the glomerulus and enter the proximal tubules as part of the glomerular filtrate. Glutathione S-conjugates produced by the renal
urine. However, they are also capable of being secreted unchanged for further metabolism, principally within the liver. Uehara et al. (1983) have described the presence of two energy-dependent
uptake systems for leukotriene E4 in rat hepatocytes which are not inhibited by GSH or GSSG. Cysteine
S-conjugates taken up within the liver are thought to be converted to the corresponding mercapturates and secreted to the plasma to be eventually excreted in the urine (Inoue et al. 1981, 1982, 1987). The
cysteine S-conjugates within the kidney may also be N-acetylated to the corresponding mercapturate and
excreted in the urine (Heuner et al. 1991) or be converted by cysteine S-conjugate β-lyases to pyruvate, ammonia, and a fragment consisting of the original electrophile modified to contain an –SH group (Section 4.17.6.3). As indicated in the foregoing
discussion, once formed in the kidney or transported to the kidney, mercapturates are excreted in the
urine. However, they are also capable of being cleaved to cysteine S-conjugates by aminopeptidases
within the kidney (see Section 4.17.5.8). The major interorgan pathways involved in the disposition of
mercapturate and mercapturate intermediates are shown in Figure 3.

Work over the last decade has established the importance of members of the ATP-binding cassette (designated ABC for human transporters and abc for rat transporters) superfamily for the cellular efflux of
GSH, GSSG, glutathione S-conjugates, and other...
conjugated metabolites. As reviewed by Cole and Deeley (2006), the ABC family in humans contains 49 members organized into seven subfamilies (A–G). Probably, the most important glutathione S-conjugate transporters are MRP1 (multidrug resistance protein 1; ABCC1) and MRP2 (ABCC2) (Cole and Deeley 2006; Nies and Keppler 2007; Sibhatu et al. 2008; Slot et al. 2008). The ABCC2 transporter is important for the transport of LTC₄ (Jedlitschky and Keppler 2002; Nies and Keppler 2007). Additional ABCC members, including long MRP6, short MRP4, MRP5, MRP8, may also be involved in GSH and glutathione S-conjugate efflux (Cole and Deeley 2006). Interestingly, there are several mechanisms by which GSH may be involved in the ABCC1-catalyzed ATP-dependent transport of various compounds directly out of the cell: (1) transport of the glutathione S-conjugate, including LTC₄, (2) GSH-stimulated egress (e.g., vincristine), and (3) GSH-stimulated efflux of the glutathione S-conjugate (e.g., the glutathione S-conjugate of nitroquinoline 1-oxide) (reviewed by Cole and Deeley 2006). The ABCC1 protein also catalyzes transport of GSSG and apigenin-stimulated GSH transport (Cole and Deeley 2006).

ABCC2 is localized to the apical membranes of various polarized cells involved in the secretion of conjugates derived from endogenous and xenobiotic substances (Nies and Keppler 2007). Nies and Keppler (2007) present evidence that ABCC2 is the major transporter for the elimination of glutathione S-, glucuronic acid- or sulfate conjugates of various toxicants and carcinogens from hepatocytes into bile, from kidney proximal tubule cells into urine, and from intestinal epithelial cells into the intestinal lumen. ABCC1 may be relatively more important in terminal detoxication in nonpolarized cell types (Nies and Keppler 2007).

Bile secretion results from the vectorial transport of solutes at the basolateral (sinusoidal) and apical (canalicular) membranes of liver parenchymal cells (Kubitz and Häussinger 2007). The transcellular transport of solutes is dependent on the hydration state of the liver. Thus, bile formation is an important
factor in liver osmoregulation. Solutes excreted into
the bile by appropriate canalicular transporters
include bile acids, glucuronides, and glutathione
$S$-conjugates (Kubitz and Häussinger 2007). Thus,
glutathione $S$-conjugate secretion plays a useful role
in liver osmoregulation. However, as noted in several
places throughout this chapter, some glutathione
$S$-conjugates (e.g., those derived from halogenated
alkenes) are toxic to the kidneys, liver, and other
organs. We suggest that the hepatotoxicity may result
in part from damage to the biliary system resulting
from conversion of the glutathione $S$-conjugate to the
cysteine $S$-conjugate followed by release of a toxic
fragment by the action of cysteine $S$-conjugate
$\beta$-lyases (see Section 4.17.6 for a discussion of the
cysteine $S$-conjugate $\beta$-lyases and their role in
bioactivation).

Awasthi et al. (2003) showed that RLIP76, a 76-
kDa Ral-binding, Rho/Rac-GAP and Ral effector
protein is a transporter of several xenobiotics and
 glutathione $S$-conjugates. RLIP bears no homology
to the ABC transporters. Glutathione $S$-conjugates of
lipid peroxidation products are transported by
RLIP76. The authors suggest that the transporter
plays an important role as a resistance mechanism
for preventing apoptosis caused by chemotherapeutic
agents and a variety of external/internal stressors,
including oxidative stress, heat shock, and
radiation (Awasthi et al. 2003). Evidently, transporters
are crucial factors in the detoxication/removal of
endogenous and exogenous electrophiles via the
mercapturate pathway.

### 4.17.4 Biotransformations of Endogenous Compounds Through the Mercapturate Pathway

#### 4.17.4.1 Deactivation/Detoxication

The most well-characterized example of the involve-
ment of the mercapturate pathway in the metabolism
of endogenous compounds relates to the sequential
transformations of leukotrienes; $LTA_4 \rightarrow LTC_4$ (glu-
thathione $S$-conjugate) $\rightarrow LTD_4$ (cysteinylglycine
$S$-conjugate) $\rightarrow LTE_4$ (cysteine $S$-conjugate) by
the consecutive actions of $LTC_4$ synthase, $GGT/\gamma$-
glutamyl leukotrienase and cysteinylglycine dipepti-
dase (Hagmann et al. 1986; Heisterkamp et al. 2008;
Murphy and Gijón 2007; Örning et al. 1986). $LTE_4$
(along with $LTC_4$ and $LTD_4$) is the slowly reacting
substance causing anaphylaxis. $LTE_4$ is a potent sti-
mulator of renal blood flow and glomerular filtration
(Allen and Gella 1990; Gubins et al. 1991). $LTC_4$
and $LTD_4$ are rapidly converted to $LTE_4$ in vivo (Moore
et al. 1992). $LTE_4$ is deactivated in vivo by conversion
to the N-acetyl derivative (mercapturate) in the kid-
ney tubules and is excreted (Fauler et al. 1991).

$LTC_4$ synthase is a member of the GST family that
is not involved in detoxication, but rather catalyzes
an important biosynthetic reaction (Ago et al. 2007). $LTC_4$
synthase is present in eosinophils, mast cells, mono-
cytes, neutrophils, and macrophages (Söderström et
al. 1988). The mouse and human $LTC_4$ synthases have
been characterized (Söderström et al. 1988). $LTC_4$
synthase is a membrane-bound enzyme (homodimer,
subunit $M_\text{r} 18 \times 10^3$) that shares some homology with
microsomal GST-1 and GST-3 (Lam 2003). The terminus
contains a protein kinase C consensus phospho-
rylation sequence, raising the possibility that the
enzyme is phosphorylated.

$LTC_4$ is formed intracellularly, but $LTD_4$ and
$LTE_4$ are formed extracellularly by the action of
the transferase and dipeptidase on the cell surface.
Most effects of the leukotrienes are exerted via inter-
actions with specific receptors on the cell surface
(Ishikawa 1993). Two cell-membrane receptors
have been well characterized, namely CysLT1
(synonyms: cysteinyl leukotriene receptor 1, cystei-
nyl leukotriene D4 receptor, LTD4 receptor) and
CysLT2 (Kanaoka and Boyce 2004). CysLTR1
belongs to the G-protein coupled receptor 1 family.
CysLT1 is the receptor that participates in cysteinyl
leukotriene-mediated bronchoconstriction of individu-
als with and without asthma. Stimulation by $LTD_4$
results in the contraction and proliferation of smooth
muscle, edema, eosinophil migration, and damage to
the mucus layer in the lung. This response is
mediated via a G-protein that activates a phosphati-
dylinositol-calcium second messenger system. The
rank order of affinities for the leukotrienes is $LTD_4$
($K_\text{d} \sim 1 \text{nM}) > LTE_4 = LTC_4$ ($K_\text{d} \sim 10 \text{nM}) > LTB_4$.
The CysLT1 receptor is widely expressed, with
highest levels in spleen and peripheral blood leuko-
cyes. Lower expression occurs in several tissues,
such as lung (mostly in smooth muscle bundles and
alveolar macrophages), placenta, small intestine, pan-
creas, colon, and heart. Selective antagonists, such as
montelukast (Singulair), zafirlukast (Accolate), and
pranlukast (Onon), are used in the treatment of
asthma, asthma-related diseases, and a few non-
asthma related diseases (Riccioni et al. 2007).

$LTC_4$ is transported from the intracellular space
to the extracellular space by an ATP-dependent
transporter with high affinity for $LTC_4$ (110 nM)
(Ishikawa 1993; Lam 2003; Schaub et al. 1991). This transporter (the GS-X pump) is identical with the GSSG transporter. (GSSG is a special case of a glutathione S-conjugate.) In addition to the kidney route for metabolism of leukotrienes mentioned above, hepatobiliary transport also occurs and this is mediated by the GS-X pump (Ishikawa 1993). It is now known that the LTC4 transporter is the MRP1/ABCC1 transporter (Km ~0.1–0.3 μmol l–1) (Cole and Deely 2006; Nies and Keppeler 2007).

Additional endogenous metabolites carried by the MRP1 carrier include the glutathione S-conjugates of prostaglandin (PG)A2, 15-deoxy-Δ12,14-PGJ2, and hydroxynonenal (Cole and Deely 2006).

### 4.17.4.2 Bioactivation

Two examples of bioactivation of endogenous compounds will be discussed here, namely catecholamines and estrogens. Catecholamines are readily oxidized to potentially toxic o-quinones (Spencer et al. 1995, 1998). These o-quinones react readily with GSH and cysteine to form catecholamine glutathione S- and cysteine S-conjugates, respectively, which may account for the low levels of GSH in the substantia nigra of Parkinson disease (PD) patients. The cysteine S-conjugates of DOPA, dopamine, and 3,4-dihydroxyphenylacetic acid are present in human brain (Spencer et al. 1998). Interestingly, the concentrations of these compounds are generally lower in many regions of human post-mortem PD brain compared to control brain. However, the concentrations of these conjugates are significantly higher in the substantia nigra of the PD patients, suggesting that these compounds may contribute to the damage in this region of the brain in PD patients (Spencer et al. 1998).

The brain has the capacity to convert the glutathione S- and cysteinyldopamine to mercapturates (Shen et al. 1996). Work by Dryhurst and coworkers have established that the mercapturates of dopamine as well as 5-S-cysteinyldopamine are potent neurotoxins. The neurotoxicity of 5-S-cysteinyldopamine may be due to their further oxidation to benzothiazine species, such as DHBT-1 (dihydrobenzothiazine-1-(2-aminooethyl)-3,4-dihydro-5-hydroxy-2H,1,4-benzothiazine-3-carboxylic acid) (Shen et al. 1996, 1997). Dryhurst and coworkers have suggested that DHBT-1 can cross the outer mitochondrial membrane and irreversibly inhibit complex I (Li and Dryhurst 1997; Li et al. 1998). The formation of DHBT-1 and other benzothiazine species requires the oxidation and intramolecular cyclization of 5-S-L-cysteinyldopamine (Li and Dryhurst 1997; Shen et al. 1997).

DHBT-1 is oxidized by an unknown enzyme in isolated mitochondria to several highly electrophilic compounds (Li and Dryhurst 1997; Li et al. 1998). These compounds are potent respiratory complex I inhibitors in isolated mitochondrial preparations (Li and Dryhurst 1997; Li et al. 1998; Shen et al. 2000). Complex I components/activity are markedly decreased in PD brain, whereas complexes II–V activities are relatively spared (Mann et al. 1992; Mizuno et al. 1989; Schapira et al. 1990). A common feature of many neurodegenerative diseases is a decline in cerebral energy metabolism. In PD brain, this decline is due in part to decreased complex I activity. However, other mitochondrial enzymes/complexes of cerebral energy metabolism (e.g., α-ketoglutarate dehydrogenase complex (KGDHC), pyruvate dehydrogenase complex (PDHC)) may also contribute to decreased energy production in neurodegenerative disease (reviewed by Gibson et al. 2005). For example, KGDHC activity is decreased in PD brain (Mizuno et al. 1994). Interestingly, DHBT-1 and its electrophilic metabolites are potent inhibitors of KGDHC (Shen et al. 2000) and PDHC (Li and Dryhurst 2001) in disrupted rat brain mitochondria, apparently via covalent modification of crucial cysteine residues.

A lifetime dose of unopposed estrogen is a significant risk factor for breast and uterine cancer (reviewed by Abel et al. 2004). Estrogens may act either by an ‘epigenetic’ pathway in which DNA is not chemically altered or they may be metabolized to products that damage DNA (Monks and Lau 1994). Catechols are major metabolic products of estrogens. They are readily oxidized to the corresponding o-quinones, which in turn are converted to GSH adducts. Monks and Lau (1994) suggested that these glutathione S-conjugates may be released from the liver ultimately to be accumulated in the kidney as the cysteine conjugates. The cysteine S-conjugates may (1) react directly with DNA, (2) generate reactive oxygen species, or (3) be further transformed to insoluble polymers (possibly involving cyclization reactions) that cause local irritation and cell proliferation (Monks and Lau 1994). More recent data from Monks and coworkers suggest that quinones derived from estriadiol and specific glutathione S-conjugates of estradiol are potent inhibitors of human GSTM1-1 and GSTA1-1 (Abel et al. 2004).
4.17.5 Metabolic Pathways Diverging from Mercapturate Biosynthesis

4.17.5.1 Deconjugation

As related to the mercapturate pathway, deconjugation is defined as the removal of the intact cysteine-containing moiety (usually as GSH) from the thioether conjugate (Stevens and Jones 1989). In general, two reactions can occur: (1) a disproportionation reaction in which GSH is converted to GSSG with concomitant elimination of the xenobiotic substituent (eqn [5]), or (2) hydrolytic cleavage of a thioester (eqn [6]).

\[
\text{GSR} + \text{GSH} \rightarrow \text{GSSG} + \text{RH} \quad (5)
\]
\[
\text{GS(CO)R} + \text{H}_2\text{O} \rightarrow \text{GSH} + \text{RCOOH} \quad (6)
\]

In other cases, the deconjugation may simply be due to reversal of the conjugation reaction; glutathione S-(and possibly cysteine S-) conjugates may then act as vehicles for the delivery of free xenobiotics and GSH throughout the body (Jean et al. 1995). Methyl isocyanate, mentioned above, is an example of a compound that is distributed throughout the body by reversible, glutathione S-conjugate formation. Other examples include unsaturated compounds, such as acrolein and crotonaldehyde. Baillie and Kassahun (1994) state that “it seems likely that many xenobiotics with carbamate, formamide, urea, or sulfonamide functionalities will undergo metabolic transformation to reactive isocyanates and that reversible conjugation to the latter short-lived, potentially toxic intermediates with GSH may play an important role in mediating the disposition and adverse effects of the parent compounds in vivo.”

4.17.5.2 Deconjugation Coupled to Reductive Dehalogenation

An example of deconjugation coupled to reductive dehalogenation is the nonenzymatic attack of GSH on 2,4-dichlorophenylacetyle chloride (eqn [7]) followed by reaction of the conjugate with GSH (eqn [8]) catalyzed by S-phencylgluthathione reductase (Kitada et al. 1985).

\[
\text{CICH}_2\text{C(O)(C}_2\text{H}_7\text{Cl}_2) + \text{GSH} \rightarrow \text{GSCH}_2\text{C(O)(C}_2\text{H}_7\text{Cl}_2) + \text{HCl} \quad (7)
\]
\[
\text{GSCH}_2\text{C(O)(C}_2\text{H}_7\text{Cl}_2) + \text{GSH} \rightarrow \text{CH}_2\text{C(O)(C}_2\text{H}_7\text{Cl}_2) + \text{GSSG} \quad (8)
\]

The rat liver enzyme catalyzes the reductive dehalogenation of o-chloroacetoephonone, but not of benzyl halides, suggesting the need for the carbonyl to stabilize a carbanion intermediate (Kitada et al. 1985). The enzyme is now known to be identical to GSTO1-1 (Board and Anders 2007). In addition to catalyzing GST reactions and S-(phenacyl)gluthathione reduction, the enzyme also catalyzes thioltransferase reactions and reduction of dehydroascorbate and monomethylarsonate (V) (Board and Anders 2007).

A second example involves vicinal dihaloalkanes. Several vicinal dihaloalkanes have been used industrially. Many of these compounds are cytotoxic and mutagenic (reviewed by Anders 2004, 2008). In the presence of GSH and a liver GST preparation these compounds are converted to glutathione S-conjugates and eventually to alkenes. Depending on the structure of the dihaloalkane, the mechanism may involve E2 elimination, substitution followed by elimination, or both (Livesey et al. 1982). In the case of 1,2-dichloroethene and 1,2-dibromoethane, reaction of the dihaloalkane with GSH yields S-(2-haloethyl)glutathione and halide ion. There is evidence for the formation of an episulfonium intermediate \((1-(\text{glutathione-S-yl})\text{thiiranium})\) from S-(2-haloethyl)glutathione as an intermediate in the formation of haloalkene (Livesey et al. 1982). S-(2-Haloethyl)glutathione may be converted to the thioether cysteine S-conjugate (S-(2-haloethyl)-L-cysteine) by the action of GGT and dipeptidases. Loss of halide ion yields an episulfonium cysteine S-conjugate \((1-(2-\text{amino-2-carboxyethyl})\text{-thiiranium})\). 1-(Glutathione-S-yl)thiiranium is thought to be the species responsible for the mutagenicity of vicinal dihaloalkanes by forming an adduct \((S-(2-(N^7\text{-guanyl})\text{-glutathione}))\) with DNA. 1-(2-Amino-2-carboxyethyl)thiiranium is thought to be the cytotoxic species (reviewed by Anders 2008).

1,2-Dibromo-3-chloropropane was used for many years as a soil fumigant to control nematodes, but was banned in the US in 1977 because it was shown to induce infertility in men and tumors in experimental animals (Clark and Snedeker 2005). The compound is converted to a glutathione S-conjugate with loss of bromide at the 1 position, but in this case deconjugation does not occur. The conjugate is converted to a thiiranium ion with loss of a second bromide ion. This thiiranium ion is implicated in the testicular toxicity of 1,2-dibromo-3-chloropropane. Hydrolysis, formation of a second thiiranium ion, and a second hydrolysis yield chloride ion and S-(2,3-dihydroxypropyl)glutathione (Anders 2008).
4.17.5.3 Hydrolytic Deconjugation

Perhaps the best-studied example occurs in the two-step glyoxalase detoxication reaction. In the first step, GSH forms an adduct with methylglyoxal (a toxic byproduct of glycolysis) yielding the glutathione S-conjugate – S-o-lactoylglutathione – in a reaction catalyzed by glyoxalase I. This enzyme is a metallo-hydrogen transfer (Creighton and Hamilton 2001). In the second step, glyoxalase II catalyzes the hydrolytic cleavage of S-o-lactoylglutathione to free GSH and D-lactate (glyoxalase II reaction) (Cordell et al. 1978; Anders 2008). GSH reacts with dichloromethane in a reaction catalyzed by several GSTs (eqn [9]) (Martin et al. 1980a,b) and dihalomethanes to formaldehyde (Ahmed and Anders 1978; Anders 2004, 2008) (eqn [10]).

\[
\text{RNH(CO)CHCl}_2 + \text{H}_2\text{O} \rightarrow \text{RNH(CO)CHO} + 2\text{HCl} \quad (9)
\]

\[
\text{CH}_2\text{X}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + 2\text{HX} \quad (10)
\]

 Dichloromethane is mutagenic in some systems and GSH is implicated in the bioactivation (Ahmed and Anders 1978; Anders 2008). GSH reacts with dichloromethane in a reaction catalyzed by GSTT1-1 to yield the corresponding glutathione S-conjugate (S-(chloromethyl)glutathione) and chloride. Hydrolysis affords S-(hydroxymethyl)glutathione. This compound is the hemithioacetal of formaldehyde and can release formaldehyde (eqn [11]). The released formaldehyde may account for the mutagenicity of dichloromethane, but model compounds also suggest a possible role for S-(chloromethyl)glutathione.

\[
\text{HCHO} + \text{GSH} \rightleftharpoons \text{GSCH}_2\text{OH} \quad (11)
\]

Formaldehyde GSH hemithioacetal is oxidized to S-(formyl)glutathione by a dehydrogenase (eqn [12]). Hydrolytic cleavage of the latter (a thioester) by S-formylglutathione hydrolase yields formic acid and GSH (eqn [13]) (Uotila and Koivusalo 1997).

\[
\text{GSCH}_2\text{OH} + \text{NAD}^+ \rightarrow \text{GSCHO} + \text{NADH} + \text{H}^+ \quad (12)
\]

\[
\text{GSCHO} + \text{H}_2\text{O} \rightarrow \text{GSH} + \text{HCOOH} \quad (13)
\]

4.17.5.4 Bis(glutathionyl) Conjugate Formation

Some xenobiotics undergo metabolic transformations that result, in part, in the formation of bis(glutathionyl) conjugates. Examples include sulfur mustards (Davison et al. 1961), melphalan (Dulik et al. 1986), hexachlorobutadiene (Jones et al. 1985), bromobenzene (Monks et al. 1985), chloroform (Pohl et al. 1981), and bromotrichloromethane (Pohl et al. 1981). The bis(conjugate) arises from an attack of a second GSH on an electrophilic center on the monoglutathionyl conjugate. The reaction may be nonenzymatic but in most cases the nature of the reaction is unknown. The mechanism of bis(glutathionyl) adduct formation with bromobenzene is complex. Bromobenzene can be converted to several products in vivo, but in one pathway in rats it is sequentially oxidized to o-bromophenol, 2-bromo hydroquinone and 2-bromoquinone. 2-Bromoquinone is subject to attack by GSH at any one of three places on the ring, yielding three possible positional isomers. These isomers can then react further with another molecule of GSH to yield 2-bromo-3,5-(diglutathionyl) hydroquinone or 2-bromo-3,6-(diglutathionyl) hydroquinone (Monks et al. 1985). In the case of hexachlorobutadiene, microsomal GST catalyzes GSH adduct formation with elimination of HCl. A second addition of GSH and elimination of HCl yields the bis(conjugate) (Monks et al. 1985).

In a similar reaction, dibromoethane is converted to the diconjugate in rat liver (eqn [14]) (Nachtomy 1970).

\[
\text{BrCH}_2\text{CH}_2\text{Br} + 2\text{GSH} \rightarrow \text{GSCH}_2\text{CH}_2\text{SG} + 2\text{HBr} \quad (14)
\]

Haloforms are oxidized in a P450-catalyzed reaction to trihalomethanol followed by dehydrohalogenation to yield dihalocarbonyl (eqns [15] and [16]). Sequential attack by two GSH equivalents yields S-haloformylglutathione (eqn [17]) and the dithiocarbonate (eqn [18]). The net reaction is given by eqn [19]. In addition, the S-haloformylglutathione conjugate may
also undergo oxidative deconjugation to yield carbon monoxide (eqn [20]) (Stevens and Anders 1979, 1981).

\[
\begin{align*}
CX_3H + O_2 + NADPH + H^+ \\
\rightarrow CX_3OH + H_2O + NADP^+ \\
CX_3OH & \rightarrow CX_3O + HX \\
GSH + CX_2O & \rightarrow GS(CO)X + HX \\
GS(CO)X + GSH & \rightarrow GS(CO)SG + HX \\
CX_3H + O_2 + NADPH + 2GSH + H^+ & \rightarrow GS(CO)SG + H_2O + NADP^+ + 3HX \\
GS(CO)X + GSH & \rightarrow CO + GSSG + HX
\end{align*}
\]

(15) (16) (17) (18) (19) (20)

### 4.17.5.5 Deamination and Transamination

DCVC is oxidized to the corresponding \( \alpha \)-keto acid by rat kidney \( \alpha \)-amino acid oxidase (Stevens et al. 1986) (eqn [21]). Conversion of a cysteine \( \alpha \)-conjugate to the corresponding \( \alpha \)-keto acid can also occur by transamination with a suitable \( \alpha \)-ketoc acid acceptor (eqn [22]) (Commandeur et al. 2000; Cooper et al. 2008a; Stevens et al. 1986).

\[
\begin{align*}
RSCH_2CH(NH_3^+)CO_2^- + O_2 + H_2O & \rightarrow RSCH_2C(O)CO_2^- + H_2O_2 + NH_4^+ \\
RSCH_2CH(NH_3^+)CO_2^- + R'C(O)CO_2^- & \rightleftharpoons RSCH_2C(O)CO_2^- + R'CH(NH_3^+)CO_2^- \\
\end{align*}
\]

(21) (22)

In theory, the \( \alpha \)-keto acid generated either by the action of \( \alpha \)-amino acid oxidase or via transamination may be reduced to the corresponding \( \alpha \)-hydroxy acid (eqn [23]). Alternatively, the \( \alpha \)-keto acid may be oxidatively decarboxylated in a reaction analogous to the PDHC reaction (eqn [24]).

\[
\begin{align*}
RSCH_2C(O)CO_2^- + NADH + H^+ & \rightleftharpoons RSCH_2CH(OH)CO_2^- + NADH^+ \\
RSCH_2C(O)CO_2^- + NAD^+ + H_2O & \rightleftharpoons RSCH_2CO_2^- + CO_2 + NADH + H^+ \\
\end{align*}
\]

(23) (24)

In some cases, oxidation of the sulfur of the conjugates in the mercapturate pathway to sulfoxides and sulfonates occurs in vivo. For example, methylsulfonylacetate, \( \beta \)-methylsulfonylthiolacetate, and methymercapturate sulfoxide are metabolites of \( \beta \)-methyl-\( \alpha \)-cysteine in rats (Barnsley 1964; Horner and Kuchinskas 1959; Sklan and Barnsley 1968). \( \alpha \)-Methylcysteine sulfoxide as well as the sulfoxides of methylthiolacetate and methylmercapturate are found in the urine of humans administered \( \beta \)-methyl-\( \alpha \)-cysteine (Mitchell et al. 1984). After administration of propachlor to normal rats, the sulfoxide of the corresponding mercapturate is a minor metabolite (Feil et al. 1981). However, after treatment with antibiotics, a considerable fraction (17%) of the dose was recovered as the sulfoxide of the mercapturate, suggesting that microbial transformations in the gut may be important routes for the metabolism of certain sulfur-containing compounds.

In some cases the sulfur of the cysteine \( \alpha \)-conjugate may be oxidized by flavin-dependent monooxygenases (FMOs), but in other cases the oxidation of the sulfur may occur after release of a thiol by a \( \beta \)-lyase reaction and thiomethylation (see the next section). In a few cases, a cysteine \( \alpha \)-conjugate sulfoxide has been shown to be a substrate of purified cysteine \( \beta \)-conjugate \( \beta \)-lyases yielding pyruvate and ammonium (Larsen and Stevens 1986; Tomisawa et al. 1986). The sulfur-containing fragment initially released in this process is presumably a sulfenic acid (RS(O)H).

Elfarra and coworkers have shown that the sulfoxide of DCVC, like DCVC itself, is nephrotoxic (Lash et al. 1994, 2003; Ripp et al. 1997; Sausen and Elfarra 1991; Sausen et al. 1993). The toxicity, however, does not appear to be due to a \( \beta \)-elimination of a sulfenic acid. Elfarra and coworkers have characterized an FMO from rat liver and kidney microsomes that has high activity toward the cysteine
S-conjugate S-benzyl-L-cysteine and that appears to be related to known flavin monoxygenase 1A1 isozymes (Ripp et al. 1997; Sausen et al. 1993).

A number of mercapturates derived from halogenated alkenes are converted to the corresponding sulfoxides by cytochrome P450 3A (but apparently not by FMOs) (Birner et al. 1998; Werner et al. 1995a,b, 1996). These sulfoxides are nephrotoxic, but the exact mechanism remains to be elucidated (reviewed by Anders 2008). Part of the nephrotoxicity of DCVC, however, may be due to sulfoxide formation in vivo and subsequent Michael addition at the terminal carbon of the vinyl group by a suitable nucleophile. Michael addition is much more favored in the case of the DCVC sulfoxide than with DCVC itself. The nucleophile may be GSH. Indeed, S-[1-chloro-2-(S-glutathionyl)vinyl]-L-cysteine sulfoxide, formed by Michael addition of GSH and elimination of HCl, was detected in the bile of rats treated with DCVC sulfoxide. There was a marked decrease of hepatic GSH which may have been depleted during the formation of the sulfoxide (Sausen and Elfarra 1991).

4.17.5.7 Thiomethyl Shunt

As already noted, many cysteine S-conjugates that contain a good leaving group are converted to pyruvate, ammonium, and a compound with a free thiol by cysteine S-conjugate β-lyases. In the thiomethyl shunt the thiol product is methylated by thiomethyltransferase (Jakoby et al. 1984; Warrander et al. 1985). S-Adenosylmethionine is the methyl donor. The resulting thiomethyl compound is then a substrate for enzymes, such as the FMOs that oxidize thioethers to sulfoxides and sulfones (Ziegler 1988). Further oxidation of the eliminated fragment could account for the formation of 14CO2 and inorganic sulfate from 14C-methyl-labeled S-methyl-L-cysteine (Horner and Kuchinskas 1959).

Finally, one other pathway that may lead to inorganic sulfate formation has been described. The cysteine conjugate is converted to the corresponding methyl sulfoxide. This compound is then reacted with GSH to yield the original glutathione S-conjugate and methane sulfenic acid (eqn [25]) which is oxidized to CO2 and sulfate (Bakke et al. 1993).

\[
RS(O)Me + GSH \rightarrow HS(O)Me + GSR \quad (25)
\]

4.17.5.8 Aminoacylase-Catalyzed Deacetylation of Mercapturic Acids

Aminoacylases catalyze the hydrolysis of mercapturates to cysteine S-conjugates and acetate. A brief discussion of these enzymes is included here because even though mercapturates are usually detoxication end-products, the mercapturate itself can sometimes be toxic, for example, in the case of mercapturates derived from halogenated alkenes (Boogard 1989). Relative toxicity of these mercapturates to the kidney and other organs depends in part on the comparative activities of cysteine S-conjugate β-lyases, cysteine S-conjugate acetyltransferase and cysteine S-conjugate aminoacylases. The halogenated cysteine S-conjugates, but not the corresponding mercapturates, are bioactivated by β-lyases (Section 4.17.6.1).

Several mammalian aminoacylases have been described, including aminoacylase I (AAI, N-acetyl amino acid hydrodrolase), aminoacylase II (AAII, asparthoacylase, N-acetyl-L-aspartate amidohydrolase), acyllysine deacylase (N'-acyl-L-lysine amidohydrolase) and aminoacylase III (Anders and Dekant 1994; Newman et al. 2007). Aminoacylase I can catalyze the deacetylation of several N-acetylated amino acids, of which N-acetyl-L-methionine is the best substrate. The enzyme also catalyzes the deacetylation of mercapturates derived from halogenated alkenes (Anders and Dekant 1994; Giardina et al. 1997, 2000; Heese et al. 1988; Kördel and Schneider 1976, 1977; Uttamsingh and Anders 1999; Uttamsingh et al. 1998a,b). Aminoacylase I activity toward halolalkene-derived mercapturates is widespread in mammalian tissues (Uttamsingh et al. 2000). The enzyme has been cloned from porcine, bovine, and rat tissues (Jakob et al. 1992; Mitta et al. 1992; Perrier et al. 2004; Raphel et al. 1999). The amino acid sequence of human aminoacylase I has been deduced from the cDNA (Mitta et al. 1993). Rat kidney, but not pig kidney, appears to contain two isoforms (Perrier et al. 2004). Pig kidney aminoacylase I shows strong sequence homology to renal dipeptidase and is now assigned to the M20 peptidase family (Liu et al. 2006). Pig kidney aminoacylase I, like the dipeptidase, is attached to membranes (Heese et al. 1988).

The bovine enzyme is a homodimer (812 amino acids per monomer) containing one zinc atom per monomer and no disulfide bonds (Kördel and Schneider 1977; Lindner et al. 2005; Liu et al. 2006).
The major physiological function is unclear, although it has been suggested to act on the terminal N-acetyl amino acids released by hydrolysis of peptides containing an N-terminal acetyl amino acid catalyzed by acylamino acid-releasing enzyme (AARE). It has not yet been possible to crystallize the enzyme. Nevertheless, Liu et al. (2006) were able to construct a useful homology model based on the known 3D structures of several members of the M20 peptide family, site directed mutagenesis and docking with N-acetyl-L-cysteine. Previous workers suggested that the zinc ion was needed to hold the enzyme in the correct configuration, but was not involved in catalysis. However, Liu et al. (2006) have presented a new model for the catalytic reaction in which Glu146 functions as a general base, accepting a proton from zinc-bound water and shuttling the proton to the leaving group facilitating breakdown of the tetrahedral intermediate. Arginine 348 helps polarize the carbonyl of the acetyl group facilitating attack by hydroxide generating a tetrahedral intermediate.

Aminoacylase III is known to exhibit a preference for N-acetyl-l-aromatic amino acids. In recent work, Newman et al. (2007) showed that recombinant mouse aminoacylase III exhibits high activity toward the N-acetyl derivatives of tyrosine, phenylalanine, tryptophan, histidine, and lysine (α positional isomer), but is inactive toward the N-acetyl derivatives of cysteine, aspartic acid, and lysine (ε positional isomer). The enzyme is, however, active toward the N-acetyl derivative of S-(benzyl)-L-cysteine, various substituted benzyl-L-cysteines, and at least eight cysteine S-conjugates derived from halogenated alkenes. Reported $V_{\text{max}}/K_m$ values for the toxic halogenated N-acetyl cysteine S-conjugates were generally similar to those of the N-acetyl derivatives of the L-aromatic amino acids, but in a few cases considerably higher (Newman et al. 2007).

Aminoacylase III has been less well studied than aminoacylase I. The mouse liver enzyme is a mixture of homodimers and homotetramers, both of which are active. A model of the dimer–tetramer interaction has recently been proposed (Ryazantsev et al. 2007).

It is interesting to note that the guinea pig kidney is especially vulnerable to the toxic effects of 2,3,5-((triglutathion-S-yl)hydroquinone. In the guinea pig, renal activity of microsomal N-acetyltransferase is lower than that in mouse and rat kidney whereas activity of the deacetylase is higher (Lau et al. 1995). The balance of N-deacetylase and N-acetylase in favor of the former in the guinea pig may contribute to the susceptibility of this species to 2,3,5-((triglutathion-S-yl)hydroquinone nephrotoxicity (Lau et al. 1995).

### 4.17.6 Cysteine S-Conjugate β-Lyases

#### 4.17.6.1 Background – Bioactivation of Halogenated Alkenes

Halogenalkenes (e.g., trichloroethylene, tetrachloroethylene, tetrafluoroethylene, and hexachloro-1,3-butadiene) are among several hundred potentially dangerous toxicants listed as present in Superfund sites in the US. Part of the US population is exposed to halogenalkenes from such sites and in the workplace (Wu and Schaum 2000), and possibly through ‘recreational’ abuse (Marjot and McLeod 1989). In experimental animals halogenalkenes, some halogenated alkanes, and dichloroacetylene are toxic, especially to the kidneys (e.g., Dekant et al. 1994; Elfarra 1993; Koob and Dekant 1991). In humans, heavy exposure to trichloroethylene is associated with an increased risk of kidney cancer (Brauch et al. 2004), PD (Gash et al. 2008), and damage to the fifth cranial (trigeminal) nerve and sensory nucleus of the brainstem (Buxton and Haywood 1967). Cranial nerve damage seems to be associated with formation of dichloroacetylene from trichloroethylene exposed to an alkaline milieu. Dichloroacetylene is an exceptionally good substrate of microsomal GSTs (eqn [2]) and hence is a good precursor of the toxic cysteine S-conjugate DCVC (Reichert et al. 1976). Damage to the trigeminal nerve may be due to the toxic effects of DCVC or to activation of latent herpes virus by the toxic insult (Cavanagh and Buxton 1989).

Trichloroethylene induces (1) aplastic anemia in cattle and (2) kidney damage in all animal species tested, including cattle (Lock et al. 1996). Depending on the structure, halogenated alkenes may be metabolized by cytochrome P450 isoenzymes (especially CYP2E1) and/or by GSTs. The ratio of the two activities varies considerably among the various halogenated alkenes. For example, hexachloro-1,3-butadiene (Wallin et al. 1988) and tetrafluoroethylene (Odum and Green 1984) are metabolized predominantly in rats via glutathione S-conjugation. However, trichloroethylene and tetrachloroethylene are metabolized mainly by cytochrome P450s, and only a small portion is metabolized by GSTs (e.g., Koob and Dekant 1991), principally GSTA1-1 (Cummings et al. 2000). Nevertheless, although the
cytochrome P450 pathway generates toxic species from trichloroethylene and tetrachloroethylene (Bull et al. 2002), a major contributor to the overall toxicity of trichloroethylene and tetrachloroethylene is the corresponding cysteine S-conjugate despite the fact that the glutathione S-conjugation pathway is quantitatively minor (Dekant 2003). The glutathione S-conjugate, cysteinylglycine S-conjugate, cysteine S-conjugate, and N-acetyl-cysteine S-conjugate (mercapturate) derived from halogenated alkenes are all nephrotoxic/hepatotoxic, and in many cases nephrocarcinogenic/hepatocarcinogenic in experimental animals (e.g., Anders 2004, 2008; Anders and Dekant 1994; Anders et al. 1988; Boogaard et al. 1989; Dekant 2001, 2003; Koob and Dekant 1991; McGoldrick et al. 2003). Some of these conjugates are also mutagenic in bacterial tester strains (Commandeur et al. 1991; Dreessen et al. 2003; Finkelstein et al. 1994; Kranendonk et al. 1997; Vamvakas et al. 1988a,b). The cysteine S-conjugate formed from trichloroethylene/dichloroacetylene (i.e., DCVC) induces expression of the proto-oncogenes c-fos and c-myc in LLC-PK1 cells (Vamvakas and Köster 1993; Vamvakas et al. 1993). The mutagenicity depends on the ability of the conjugates to generate chemically reactive thiols – GGT and cysteine S-conjugate β-lyases are key enzymes in this process. As noted above, the mercapturates derived from the halogenated alkenes are toxic because they can be hydrolyzed back to the corresponding cysteine S-conjugate by aminocylases (e.g., Utramsingh et al. 2000). Thus, the mercapturate pathway enzymes together with aminocylases and cysteine S-conjugate β-lyases contribute to the bioactivation of halogenated alkenes. (For reviews, see Anders 2004, 2008; Cooper 1998; Cooper and Pinto 2008; Dekant 2003; Dekant et al. 1994.) The reactive fragments generated from haloalkene cysteine S-conjugates by the action of cysteine S-conjugate β-lyases are especially cytotoxic in experimental animals to the proximal renal tubules, in particular to the S3 (pars recta) region and, to a lesser extent, the S1, S2 (pars convoluta) region (e.g., Jones et al. 1988) and to isolated human proximal tubules (Chen et al. 1990).

4.17.6.2 Possible Repair Mechanisms

Cytosolic rat kidney cysteine S-conjugate β-lyase (or more accurately GTK) and its mRNA were shown to be elevated in the kidneys of rats given a single nonnephrotoxic dose of N-acetyl-S-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine (MacFarlane et al. 1993). However, nephrotoxic doses resulted in reduction of the total lyase activity (MacFarlane et al. 1993). This reduction may result in part from the fact that turnover of cysteine S-conjugates derived from halogenated alkenes can result in syncatalytic inactivation of cysteine S-conjugate β-lyases (Section 4.17.6.8). A reduction in the lyase activity could be a protective mechanism as long as the normal metabolic function of the lyase is not compromised too severely.

It has been shown that a lethal dose of DCVC in mice leads to inhibition of cell division, inhibition of renal repair and acute renal failure (Korrapati et al. 2005; Vaidya et al. 2003a,b). However, sub-lethal (priming) doses are autoprotective by stimulating cell division and renal repair (Korrapati et al. 2006). Acute renal failure in humans is associated with high morbidity and mortality rates. However, it is potentially reversible if the patients are free of life-threatening complications. Korrapati et al. (2007) have pointed out that the pathophysiology of DCVC-induced acute tubular necrosis followed by acute renal failure in mice and clinical acute renal failure in patients is similar. These authors have begun a proteomic study of this clinically relevant mouse model of priming with low doses of DCVC to afford later autoprotection to the kidney. Whole kidney homogenates from treated and untreated mice were analyzed on 2D gels and spots were identified by mass spectrometry. Eighteen common proteins were shown to be greatly altered in the treated mice, including some enzymes associated with energy metabolism and stress response proteins. Although the proteomic approach to understanding renal disease is in its infancy, this model suggests that biomarker development will be useful in a variety of toxicological paradigms (Korrapati et al. 2007).

4.17.6.3 The Cysteine S-Conjugate β-Lyase Reaction

Colucci and Buyske (1965) showed that benzothiazole-2-sulfonamide is converted in rats, rabbits, and dogs, not only to the corresponding mercapturate but also to 2-mercaptobenzothiazine in which the sulfur of the mercaptan moiety is derived from GSH. Later, Schultz and coworkers suggested that the toxicity of DCVC is associated with a ‘C-S’ lyase reaction and formation of a reactive sulfur-containing species that adds to macromolecules, including proteins and nucleic acids (e.g., Anderson and Schultz 1965;
Bhattacharya and Schultze 1967, 1972). It was known for many years that several electrophilic xenobiotics (e.g., phenacetin, acetaminophen, N-hydroxy-2-acetylaminofluorene) are thiomethylated in vivo. Thiomethylation was assumed to involve a sulfoxide compound derived from methionine. However, Chatfield and Hunter (1973) showed that conversion of 2-acetamido-4-chloromethylthiazole to 2-acetamido-4-methylthiomethylthiazole in rats involves the mercapturate pathway.

The thiomethylation pathway was elucidated by Tateishi and colleagues (1978a,b). When bromazepam was administered to rats, the corresponding mercapturate and 6'-methylthiobromazepam were identified in the bile (Tateishi et al. 1978a). Tateishi et al. (1978a) also showed that the thioether bond of the cysteine S-conjugates of 2,4-dinitrobenzene and bromobenzene is readily cleaved by an enzyme present in rat liver cytosol. Incubation of the purified enzyme with S-(2,4-dinitrophenyl)cysteine yielded pyruvate, ammonium and 2,4-dinitrobenzenethiol. Incubation of the thiol product with a microsomal thiomethyltransferase and S-adenosylmethionine yielded the corresponding thiomethyl compound. Tateishi et al. (1978a,b) coined the term ‘cysteine conjugate β-lyase’ to describe an enzyme that catalyzes β-elimination from a cysteine S-conjugate. The net cysteine S-conjugate β-lyase-catalyzed reaction is shown in eqn [26].

\[ \text{XSH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{C}(\text{O})\text{CO}_2^- + \text{NH}_4^+ + \text{XSH} \] (26)

The actual products of the enzyme-catalyzed reaction are XSH and aminoacrylate \([\text{CH}_2=\text{C}(\text{NH}_3^+)^-\text{CO}_2^-]\). The latter undergoes nonenzymatic tautomerization to the α-amino acid \([\text{CH}_3\text{C}(=\text{NH}_3^+)^-\text{CO}_2^-]\) and subsequent hydrolysis to pyruvate \([\text{CH}_3\text{C}(\text{O})\text{CO}_2^-]\) and ammonium. When the eliminated sulfur-containing fragment (XSH) is stable (i.e., does not contain electrophilic moieties that cause XSH to be converted to an extremely reactive electrophile), the –SH group may be methylated in the case of the sulfur-containing fragment derived from S-(2,4-dinitrophenyl)cysteine (Tateishi et al. 1978a) (Figure 1, reaction 8; Section 4.17.5.7). The thiomethylated compound may also be oxidized to the sulfoxide as described in Section 4.17.5.6. Alternatively, XSH may be glucuronidated as in the case of 2-benzothiazole derived from benzothiazolyl L-cysteine (BTC) (Elfarra and Hwang 1990) (Figure 1, reaction 9). Mercapturates and S-glucuronides are readily excreted. Thiomethyl derivatives may be excreted intact or further oxidized. The fate of chemically reactive XSH fragments is discussed in Section 4.17.6.7.

### 4.17.6.4 Cysteine S-Conjugate β-Lyases in Enteric Bacteria

Several studies have shown that many enteric bacteria contain cysteine S-conjugate β-lyases (Bernström et al. 1989; Larsen and Bakke 1983; Larsen and Stevens 1986; Larsen et al. 1983; Saari and Schultze 1965; Suzuki et al. 1982; Tomisawa et al. 1984; Wakabayashi et al. 2004). Escherichia coli tryptophanase (a ‘true’ β-lyase) and a cysteine S-conjugate β-lyase in an extract of Eubacterium limosum were shown to catalyze β-elimination reactions with the cysteine S-conjugates of E-2-hexenal and 2-hexenol (Wakabayashi et al. 2004). The cysteine S-conjugate β-lyases purified from Fusobacterium necrophorum (Larsen et al. 1983) and F. varium (Tomisawa et al. 1984) have been partially purified and characterized. The enzymes have \(M_r\) values of \(228 \times 10^3\) and \(70 \times 10^3\), respectively, and require pyridoxal 5'-phosphate (PLP) as a coenzyme. The enzyme from F. necrophorum catalyzes the C-S cleavage of the cysteine S-conjugate of propachlor, BTC, and 1,2-dihydro-1-hydroxy-2-cysteinylnaphthalene. The propachlor conjugate was shown to be converted to N-isopropylacetanilide, pyruvate, and ammonium. The enzyme from F. varium is most active with aromatic cysteine S-conjugates, but some activity is noted with simple alkyl conjugates. A highly purified cysteine S-conjugate β-lyase was obtained from E. limosum (Larsen and Bakke 1983). The enzyme has an \(M_r\) of about \(75 \times 10^3\) and is composed of two identical subunits. The enzyme is active with the cysteine S-conjugate of propachlor, trans-9-hydroxy-10-(S-(l-cysteinyl))-9,10-dihydrophenanthrene, BTC, and DCVC. It is also moderately active with the simple alkyl cysteine conjugate S-ethyl-L-cysteine. The enzyme does not require added PLP, but is inhibited by carbonyl reagents suggesting the presence of a carbonyl-containing cofactor. Of interest is the finding that the enzyme is active with cystathionine, djenkolic acid, lanthionine, and cystine, suggesting that the enzyme is similar to cystathionine β- and γ-lyases. In contrast, the F. necrophorum and F. varium lyases do not exhibit activity with cystathionine. Bernström et al. (1989) showed that rat fecal contents and the purified E. limosum enzyme catalyze the conversion of the cysteine S-conjugate LTE₄ to 5-hydroxy-6-mercapto-7,9-trans-11,14-cis-eicosatetraenoic acid.
Interestingly, the enzyme can catalyze the conversion of \( \beta \)-chloroalanine to pyruvate and ammonia, but it is not inactivated in the process. Many PLP-containing enzymes that catalyze this elimination reaction are slowly inactivated by aminoacylrate intermediate(s) formed during the elimination reaction (Section 4.17.6.8). Finally, the \( E. \ limosum \) enzyme exhibits high activity with the \( S \)-oxide of propachlor (Larsen and Stevens 1986). The eliminated fragment is presumably a sulfenic acid –RS(O)H.

Gut bacteria are known to be important in catalyzing biotransformations of certain xenobiotics, including conjugate formation with GSH (Bakke et al. 1981). The fact that cysteine \( S \)-conjugate \( \beta \)-lyase activity is widespread in enteric bacteria and that a major portion of cysteine \( S \)-conjugates are excrated into the bile suggests that the gut flora may be important for the incorporation of sulfur into some xenobiotics (Larsen and Stevens 1986). Moreover, it seems that the cysteine \( S \)-conjugate \( \beta \)-lyases of the enteric bacteria have different substrate specificities than do the mammalian enzymes. For example, the cysteine \( S \)-conjugate of propachlor is a substrate for the enteric enzymes, but germ-free rats do not metabolize this compound (Bakke et al. 1981). As noted above, certain sulfhydryl-containing fragments, such as that eliminated from DCVC, are exceptionally reactive and are likely to act locally. Whether cysteine \( S \)-conjugates, whose elimination products are very reactive, damage the gut remains to be determined. Additionally, whether more stable thiol products generated from cysteine \( S \)-conjugates can be transported from the gut to other regions to exert toxic effects also remains to be evaluated (Larsen and Stevens 1986).

### 4.17.6.5 Cysteine \( S \)-Conjugate \( \beta \)-Lyases in Mammals

Thirty years ago, Tateishi and coworkers (1978b) obtained a highly purified preparation of a cysteine \( S \)-conjugate \( \beta \)-lyase from rat liver, but they did not identify it. The same group also obtained a highly purified preparation of a PLP-dependent cysteine \( S \)-conjugate \( \beta \)-lyase from human liver (Tomisawa et al. 1986). The human liver enzyme is active with \( S \)-arylcysteines but not with \( S \)-alkylcysteines. The enzyme, which catalyzes stoichiometric formation of pyruvate, ammonium, and \( p \)-bromophenylmercaptan from \( S(p \text{-bromophenyl}) \text{-L-cysteine} \) has a pH optimum of 8.5 and has a native \( M_r \) of \( \sim 88 \times 10^3 \). Interestingly, the enzyme is nine times more active with \( S \)-phenylcysteine sulfoxide than with \( S \)-phenylcysteine. The identity of this enzyme was also not established. Moreover, the specific activity of the purified enzyme is relatively low (12.3 nmol min\(^{-1}\) mg\(^{-1}\) with \( S(p \text{-bromophenyl}) \text{-L-cysteine} \) and cysteine \( S \)-conjugates of halogenated alkenes were not tested as substrates.

In the mid-1980s, kynureninase (Stevens 1985) and GTK (identical to kynurenine aminotransferase isozyme I, KAT I) (Stevens et al. 1986) were identified as major cysteine \( S \)-conjugate \( \beta \)-lyases of rat liver and kidney cytosol, respectively (DCVC as substrate). Over the last 20 years many more mammalian cysteine \( S \)-conjugate \( \beta \)-lyases have been identified (Table 1). All are PLP-containing enzymes and include (1) the cytosolic enzymes kynureninase, GTK/KAT I, cytosolic aspartate aminotransferase (cytAspAT), alanine aminotransferase (AlaAT), and cysteine branched-chain aminotransferase (BCAT\(_c\)), and (2) the mitochondrial enzymes mitochondrial aspartate aminotransferase (mitAspAT), mitochondrial branched-chain aminotransferase (BCAT\(_m\)), alanine-glyoxylate aminotransferase isozyme II (AGAT II), and GABA aminotransferase (reviewed by Cooper and Pinto 2006, 2008). In addition, high-\( M_r \) \( \beta \)-lyases occur in both cytosolic and mitochondrial fractions of rat kidney and, to a lesser extent, rat liver. High-\( M_r \) forms in rat kidney cytosol and mitochondria contain GTK and mitAspAT, respectively (manuscript in preparation). Of all the mammalian cysteine \( S \)-conjugate \( \beta \)-lyases identified thus far, human GTK has the highest inherent specific activity (Table 1; Cooper and Pinto 2008).

### 4.17.6.6 Cysteine \( S \)-Conjugate \( \beta \)-Lyases in Other Organisms

Fungi are known to contain GST activity (Ando et al. 1988; Dowd and Sheehan 1999) and cysteine \( S \)-conjugate \( \beta \)-lyase activity (Hafsah et al. 1987; Shimomura et al. 1992). For example, \( Mucor circinelloides \) (formerly \( M. javanicus \)) converts 2,4-dichloro-1-nitrobenzene to the corresponding glutathione \( S \)-conjugate, cysteine \( S \)-conjugate, and mercapturate as well as to 5-chloro-2-nitrobenzenethiol (Hafsah et al. 1987; Shimomura et al. 1992). Evidently, the role of fungi in transforming environmental xenobiotics through the mercapturate and \( \beta \)-lyase pathways needs to be further evaluated.

Three species of parasitic helminths have been shown to contain cysteine \( S \)-conjugate \( \beta \)-lyase activity (Adcock et al. 1999). A cysteine \( S \)-conjugate \( \beta \)-lyase was purified from the tapeworm \( Moniezia \)
expansa and shown to purify with an enzyme that exhibited AspAT activity (Adcock et al. 2000).

3-Mercaptohexanol is an odor detected in passion fruit and in Sauvignon blanc wines (Wakabayashi et al. 2004). 3-Mercaptohexanol was suggested to be derived from the cysteine S-conjugate of E-2-hexenal (Wakabayashi et al. 2004). A β-lyase reaction with this compound will release 3-mercaptohexenal, which may be reduced to the corresponding alcohol. An alternative route may occur, namely reduction of the cysteine S-conjugate of E-2-hexenal to the cysteine S-conjugate of 2-hexenol, followed by a cysteine S-conjugate β-lyase-catalyzed elimination reaction to yield directly 3-mercaptohexenol. Both pathways are feasible. A series of alkanoates of 3-methyl-3-sulfanylbutan-1-ol ((CH₃)₂C(SH)CH₂CH₂OC(O)R) and a series of alkanoates of 3-sulfanylhexan-1-ol (CH₃CH₂CH₂CH₂CH₂CH₂OC(O)R), have recently been detected in the fruit peel of Poncirus trifoliata (a close relative of citrus) (Starkenman et al. 2007). The authors suggested that the origin of these volatile sulfur-containing compounds is via β-lyase reactions on cysteine S-conjugates.

The odor of human sweat may be enhanced by the action of skin bacteria. 3-Sulfanylhexan-1-ol, along with 2-methyl-3-sulfanylbutan-1-ol, 3-sulfanylpentan-1-ol and 3-methyl-3-sulfanylhexan-1-ol, have been identified as odiferous compounds in human sweat (Natsch et al. 2004). It was suggested that the nonodiferous precursors of the sulfanylalkanols are cysteine S-conjugates. In agreement with this hypothesis, the authors showed that cysteine S-conjugates of sulfanylalkanols are substrates of a cysteine S-conjugate β-lyase cloned from the skin bacterium Corynebacterium striatum Ax20 present in human axillary secretions (Natsch et al. 2004). The enzyme responsible was identified as cystathionine β-lyase.

Alliinase, an enzyme found in garlic and other allium vegetables, catalyzes the β-elimination of allylsulfenic acid from 1-alliin – a cysteine S-conjugate sulfoxide (eqns [27] and [28]) (Cooper and Pinto 2005, and references quoted therein). Allylsulfenic acid is extremely reactive and forms the anhydride allicin in an aqueous medium. Allicin reacts with cysteine to form a series of cysteine S-conjugates. Several of these cysteine S-conjugates are β-lyase substrates of cystathionine γ-lyase (Cooper and Pinto 2005). In some cases the eliminated product is a persulfide, which may be a source of sulfane (S¹ or S²) sulfur, contributing to the anticancer properties of garlic (Pinto et al. 2006).
4.17.6.7 Reactive Sulfur-Containing Fragments Generated by \( \beta \)-Lyase Reactions Involving Halogenated Alkene Cysteine S-Conjugates

A few examples will be provided here. For a more detailed coverage see Anders (2008). The cysteine S-conjugate \( \beta \)-lyase reaction with DCVC (1) gives rise to pyruvate (2), ammonium and a sulfur-containing fragment that has the theoretical structure 1,2-dichloroethylenethiolate (3) (Figure 4). However, 1,2-dichloroethylenethiolate is extremely unstable and may tautomerize to chlorothioacetyl chloride \([\text{ClC}(-\equiv\text{S})\text{CH}_2\text{Cl}]\) (not shown) or spontaneously lose \( \text{Cl}^- \) to form the highly reactive chlorothioketene (4) (Anders 2004, 2008; and references cited therein).

The chlorothioketene reacts with a variety of nucleophiles (Nu:) to generate a thioacylated adduct (5). In biological systems the nucleophile may be macromolecules, such as nucleic acids (Müller et al. 1998) and proteins (Eyre et al. 1995). This chemistry provides an explanation for the previous finding of Anderson and Schultze (1965), who as alluded to above, showed covalent incorporation of a sulfur-containing fragment from DCVC into macromolecules via the action of ‘C-S’ lyases.

Cysteine S-conjugate \( \beta \)-lyase-catalyzed bioactivation of \( S-(1,1,2,2\text{-tetrafluoroethyl})\text{-l-cysteine (TFEC, the cysteine S-conjugate of tetrafluoroethylene) is shown in Figure 5. The \( \beta \)-lyase reaction with TFEC (1) results in the formation of pyruvate (2), ammonium
and 1,1,2,2-tetrafluoroethanethiolate (3). 1,1,2,2-Tetrafluoroethanethiolate is very reactive, losing $\text{F}^-$ to generate difluorothioacetyl fluoride (4), which thioacylates nucleophiles. $\text{^{15}S}$-Labeling studies performed in vivo show that phosphatidylethanolamine is a major nucleophile thioacylated in mitochondria by the action of cysteine $\beta$-conjugate $\beta$-lyases on TFEC (Hayden et al. 1992). The $\varepsilon$-amino group of protein lysyl moieties are also especially vulnerable to thioacylation by 4 (Fisher et al. 1993; Harris et al. 1992; Hayden and Stevens 1990; Hayden et al. 1991).

Bromine-containing 1,1-difluoroalkene-derived cysteine $\beta$-conjugates are nephrotoxic, but unlike the nonbrominated analogues, these compounds are also mutagenic in the Ames test (Finkelstein et al. 1994). Initial studies with the cysteine $\beta$-conjugate of 1-bromo-2-chloro-1,1-difluoroethene with a rat kidney homogenate and a pyridoxal model system showed formation of glyoxylate as a product (Finkelstein et al. 1995). The eliminated sulfur-containing fragment (1,1-difluoro-2-bromo-2-chloroethanethiolate) was suggested to undergo conversion to a three membered ring structure (3-chloro-$\alpha$-thiolactone) with the loss of $\text{F}^-$ and $\text{Br}^-$. The 3-chloro-$\alpha$-thiolactone was then proposed to undergo conversion to glyoxylate by hydrolysis and loss of $\text{Cl}^-$ and $\text{H}_2\text{S}$ (Finkelstein et al. 1995). Later work from the same group using $o$-phenylenediamine as a trapping agent suggested that thiirene [2,2-difluoro-3-chlorothiane] was a likely intermediate in the decomposition of 1,1-difluoro-2-bromo-2-chloroethanethiolate (Anders 2005; Finkelstein et al. 1996).

Sevoflurane [(F,C)$_2$COCH$_2$F] is a widely used anesthetic which may lose HF in the anesthetic circuit to generate 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A), which is nephrotoxic to rats (Anders 2005). In the rat, Compound A can form two glutathione $\beta$-conjugates, which are eventually converted to the corresponding cysteine $\beta$-conjugates. These cysteine $\beta$-conjugates are substrates of cysteine $\beta$-lyases giving rise to 2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropanethiol and 2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenethiol, both of which are converted to 2-(fluoromethoxy)-3,3,3-trifluorothio-1-propenyl fluoride. Hydrolysis and loss of HF and $\text{H}_2\text{S}$ yields 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid. Finally, loss of HF and HCHO yields trifluoroacetic acid. Evidence suggests that a similar pathway for the metabolism of Compound A exists in humans. The pathway suggests that cysteine $\beta$-conjugate $\beta$-lyases are involved in the bioactivation of Compound A in humans, but this hypothesis has been criticized. Moreover, there are no confirmed cases of sevoflurane-induced kidney damage in humans. See Anders (2005) for a comprehensive review and original references.

### 4.17.6.8 Catalytic Mechanism of the Cysteine $\beta$-Conjugate $\beta$-Lyase Reaction and Syncatalytic Inactivation

Table 1 reveals that, with the possible exception of cystathionine $\gamma$-lyase, no mammalian cysteine $\beta$-conjugate $\beta$-lyase identified thus far catalyzes a $\beta$-lyase reaction as its ‘natural’ physiological reaction. Although cystathionine $\gamma$-lyase normally catalyzes a $\gamma$-elimination reaction, it can also catalyze $\beta$-elimination reactions that may be physiologically relevant. For example, rat liver cystathionine $\gamma$-lyase catalyzes (1) the formation of $\text{S}$-mercaptot-l-cysteine from $l$-cystine (Cavallini et al. 1960), (2) $\beta$-elimination of alkane thiols from several nonhalogenated cysteine $\beta$-conjugates containing alkyl groups attached to the sulfur (Tomisawa et al. 1988), and (3) $\beta$-elimination of alkyl/allyl thiols/persulfides from various alkyl/allyl cysteine $\beta$-conjugates present in garlic extracts (Cooper and Pinto 2005; Pinto et al. 2006). Except for kynureninase, the remaining mammalian cysteine $\beta$-conjugate $\beta$-lyases listed in Table 1 are aminotransferases.

Most PLP-catalyzed reactions involving amino acids proceed through a quinonoid intermediate formed between substrate and active site PLP (Metzler 2001). In the normal physiological reaction catalyzed by kynureninase a quinonoid intermediate is converted to an enamine intermediate with resonance stabilized partial carbanion character at the terminal carbon (eqn [29]) (where $\text{R} = \text{remainder of the quinonoid intermediate}$) (Stevens 1985). However, when DCVC binds to the active site a $\beta$-elimination is catalyzed as a result of the strongly electron-withdrawing moiety attached at the sulfur. This results in the formation of an enamine with resonance stabilized partial carboxylation character (eqn [30]) (where $\text{R}^+ = \text{protonated pyridine form of the intermediate and R is a quinonoid intermediate}$). In the former case alanne $\text{[CH}_3\text{CH(CO}_2^-\text{)NH}_3^+]$ is released from the active site. In the latter case, aminoacrylate $\text{[CH}_2\text{=C(CO}_2^-\text{)NH}_3^+]$ is released, which, as noted above, is nonenzymatically converted to pyruvate and ammonium.
When transamination competes with the β-lyase reaction, an α-keto acid (e.g., KMB, phenylpyruvate) substrate (or PLP) must be present in the reaction mixture to maintain the β-elimination reaction. A half-transamination reaction will convert the PLP coenzyme to its pyridoxamine 5'-phosphate (PMP) form, which cannot catalyze a β-lyase reaction. The α-keto acid substrate forms a Schiff base with PMP that is converted to the corresponding amino acid and PLP. The PLP form of the enzyme can then catalyze another round of the β-lyase reaction (Cooper 1998; Stevens et al. 1986).

β-Elimination reactions catalyzed by aminotransferases have been known for at least 40 years. For example, pig heart cytAspAT catalyzes β-elimination reactions with β-chloroalanine, serine O-sulfate and serine O-phosphate (John and Fasella 1969; Morino et al. 1974; Ueno et al. 1982). The ratio of transamination to β-elimination depends in part on the electron-withdrawing properties (nucleofugacity) of the group attached at the β-position of the amino acid substrate. With β-chloroalanine and serine O-sulfate only β-elimination can be detected in the presence of cytAspAT (Morino et al. 1974; Ueno et al. 1982). Several aminotransferases have been shown to catalyze a β-elimination reaction with TFEC (Table 1). In most cases, transamination was shown to compete with β-elimination. Presumably –SCF₂CF₂H is a less effective nucleofuge than is –Cl. However, the β-elimination to transamination ratio also depends on the enzyme. For example, for mitAspAT with TFEC as substrate the ratio is >100 (Cooper et al. 2002b), but approximately 2:1 for rat kidney GTK (Commandeur et al. 2000). Human BCATₘ can catalyze a β-lyase reaction with TFEC, but transamination cannot be detected (Cooper et al. 2003).

In many cases, β-elimination catalyzed by aminotransferases leads to eventual syncatalytic inactivation. The inactivation of cytAspAT by β-chloro-L-alanine, however, can be decreased by inclusion in the reaction mixture of a Michael acceptor such as thiosulfate (Cavallini et al. 1973) or β-mercaptoethanol (Adams et al. 2005). The products obtained are L-cysteine S-sulfonate and 3-(2-hydroxyethyl)-L-cysteine, respectively. The inactivating species is evidently aminoacrylate. The aminoacrylate may be free in solution as suggested by Cavallini et al. (1973) or covalently bound to PLP coenzyme (Adams et al. 2005). The latter is suggested by retention of configuration at the α-carbon of product 3-(2-hydroxyethyl)-L-cysteine when aminoacrylate is trapped with β-mercaptoethanol (Adams et al. 2005).

It was originally suggested that inactivation of cytAspAT by β-chloro-L-alanine is due to modification of a lysine residue by aminoacrylate (Morino et al. 1974). However, Ueno et al. (1982) presented evidence that the inactivation in the presence of serine O-sulfate is due to formation of the pyruvate–PLP aldol product. More recently, we showed that rat liver mitAspAT is syntactically inactivated by β-chloroalanine and TFEC on average after ~3850 and ~2700 turnovers per enzyme monomer, respectively (Cooper et al. 2002b). We ascribed this difference to the production of one reactive species (aminoacrylate or aminoacrylate bound as Schiff base) in the case of β-chloroalanine, but two reactive species in the case of TFEC (aminoacrylate and –SCF₂CF₂H). The eliminated sulfur-containing fragment is chemically very reactive (previous section). Previous work has shown that the ε amino groups of lysine residues are susceptible to thioaclylation (formation of RNHC(S)CF₂H₃, where R = lysine residue; Fisher et al. 1993; Harris et al. 1992; Hayden and Stevens 1990; Hayden et al. 1991). We have begun to investigate the mechanism of inactivation of mitAspAT by TFEC. We have noted lysine thioaclylation as expected, but have also detected –CF₂CF₂H linkage to a lysine residue and aminoacrylate addition to a cysteine residue (Cooper and Pinto 2008). Curiously, both human BCATₘ and BCATₑ can catalyze β-elimination with TFEC, but both enzymes are rapidly inactivated. Inactivation occurs on average ~170–280 and 40–50 turnovers per subunit for BCATₘ and BCATₑ, respectively (Cooper et al. 2003). On the other hand, both rat kidney GTK and human GTK are resistant to inactivation by β-lyase substrates. Human GTK has an unusual ‘crown’ of aromatic amino acid residues in the substrate binding pocket, which may account for the interaction with neutral amino acids, including cysteine S-conjugates (Rossi et al. 2004). This arrangement may prevent access of reactive fragments to susceptible groups within the vicinity of the active site. Evidently, the relative ease of syncatalytic inactivation by β-lyase substrates varies greatly among the aminotransferases, and...
probably depends on ease of access of reactive fragments to susceptible residues in the active site.

4.17.7 Major Cysteine S-Conjugate β-Lyases of Mammalian Tissues

As noted above, the first two mammalian cysteine S-conjugate β-lyases to be positively identified were kynureninase (Stevens 1985) and GTK (Stevens et al. 1986). Because GTK is (1) particularly prominent in vitro as a β-lyase with toxic cysteine S-conjugates such as TFEC (Table 1), (2) the enzyme is present in rat and human kidney proximal tubules (Jones et al. 1988), and (3) the proximal tubules are especially prone to damage from toxic halogenated cysteine S-conjugates, it was quickly assumed that GTK is the cysteine S-conjugate β-lyase. Indeed, the enzyme is given this name in the human genome directory. The gene for the human enzyme CCBL1(cysteine conjugate β-lyase 1) is located on chromosome 9 (Perry et al. 1995).

Naming GTK as cysteine S-conjugate β-lyase is unfortunate because as shown in Table 1 other PLP-dependent enzymes may also contribute to the formation of reactive fragments from nephotoxic cysteine S-conjugates. In the following sections we focus on GTK and mitAspAT as major cysteine S-conjugate β-lyases.

4.17.7.1 Glutamine Transaminase K/Kynurenine Aminotransferase I

The ability of glutamine to participate in enzyme-catalyzed transamination was first discovered by Meister et al. in 1952. Subsequently, Cooper and Meister (1974, 1981) discovered that rat tissues contain at least two glutamine transaminases, a kidney type named glutamine transaminase K (GTK, highest specific activity in kidney, but well represented in other tissues) and a liver type named glutamine transaminase L (GTL, predominantly in liver, with only low activity in other tissues). Very little work has been carried out on GTL. On the other hand GTK has been extensively studied.

As summarized by Cooper (2004), GTK in vitro exhibits a broad specificity toward t-amino acids and α-keto acids. In general, the enzyme has a preference for t-glutamine, t-methionine (and many other sulfur-containing amino acids), l-phenylalanine (and some other aromatic amino acids), and the corresponding α-keto acids. As noted above, cysteine S-conjugates are also substrates of rat kidney GTK. Interestingly, l-selenocysteine S-conjugates are more active aminotransferase and β-lyase substrates of rat kidney GTK than are the corresponding cysteine S-conjugates, in most cases by an order of magnitude or more (Commandeur et al. 2000). As a consequence, GTK has been suggested to be a possible target for selenium-containing chemopreventive drugs (Commandeur et al. 2000). l-Glutamine is the major amino acid substrate in vivo. The product of glutamine transamination (i.e., α-ketoglutarate; αKGM) is rapidly removed by cyclization to a lactam and/or conversion to α-ketoglutarate in a reaction catalyzed by ω-amidase. Transamination is therefore ‘pulled’ in the direction of glutamine utilization. Major roles of GTK are to close the methionine salvage pathway by transamination of α-keto-γ-methylbutyrate (KMB), salvage the α-keto acid analogues of essential amino acids, and possibly to generate cyclic sulfur-containing imines (reviewed by Cooper 2004).

Perry et al. (1993) reported the amino acid sequence deduced from the cDNA of an enzyme from rat kidney to which they assigned the name cysteine S-conjugate β-lyase. The authors showed that the monomer has a $M_\text{r}$ of $47 \times 10^3$ and is composed of 423 amino acid residues. A conserved PLP binding site is present. Shortly thereafter, Mosca et al. (1994) deduced the amino acid sequence for rat kidney KAT I from the cDNA and expressed the enzyme in COS-1 cells. The expressed enzyme had both KAT and GTK activities. The two reported sequences were almost identical. The only difference was Ala for Arg at residue 107 and Val for Ile at residue 177 in the sequence deduced by Mosca et al. (1994) versus that deduced by Perry et al. (1994). Mosca et al. (1994) assumed that rat kidney KAT is identical to cysteine S-conjugate β-lyase and to GTK and that the differences between the sequences obtained by the two groups is due to variability in the strain of rats used.

GTK occurs in the cytosolic and mitochondrial fractions of rat tissues (Cooper and Meister 1981). Interestingly, the mitochondrial form of GTK/KAT I is identical to the cytosolic form except that the former contains an additional stretch of 32 amino acids at the N-terminus (Malherbe et al. 1995). To what extent the 32-amino acid leader sequence is cleaved after entry into the mitochondria is not clear. Apparently, the presence of cytosolic and mitochondrial forms is due to the presence of two mRNAs derived from the same gene coding for proteins,
respectively, with and without mitochondria-targeting leader sequences (Malherbe et al. 1995; Mosca et al. 2003).

Mosca et al. (2003) cloned four cDNAs from a kidney cDNA library containing several different 5' UTRs. One of the transcripts (+14KAT1 cDNA) contains an alternative translation initiation site. The authors showed that several KAT I mRNAs are expressed in a ubiquitous manner, whereas +14KAT1 mRNA is present only in kidney. The KAT I mRNAs exhibit different in vitro translational efficiencies, which correlate with the levels of enzyme activity in transfected Cos-1 cells. The findings are in accord with the predicted accessibility of the ribosomal binding sites of the different mRNAs (Mosca et al. 2003).

Abraham and Cooper (1996) cloned and sequenced a rat kidney GTK (subunit $M_r \sim 48\,500$). The amino acid sequence of GTK deduced by Abraham and Cooper is $>90\%$ identical to that deduced by Perry et al. (1993) and Mosca et al. (1994). The reason for the complex regulation of GTK/KAT I protein levels in rat tissues via different mRNAs with varying translational efficiencies, and possibly by two different genes, remains to be elucidated.

Human GTK was purified from kidney tissue (Lash et al. 1990b). Subsequently, human GTK was cloned and sequenced (Goldfarb et al. 1996; Perry et al. 1995). The deduced amino acid sequence of GTK deduced by Perry et al. is $92\%$ identical to that deduced by Lash et al. Human GTK is a homodimer (subunit $M_r \sim 47\,900$) containing one PLP per monomer (Rossi et al. 2004). Crystallized rhGTK has the prototypical fold of an aminotransferase of subgroup I (Rossi et al. 2004). As is typical of aminotransferases, the active site of rhGTK is open in the absence of substrate, but changes to a more closed arrangement upon covalent attachment of the amino acid substrate to the PLP-coenzyme. As noted above, the active site has a high degree of hydrophobicity that allows binding of large noncharged amino acids and cysteine $S$-conjugates.

To our knowledge a possible mitochondrial form of human GTK has not been considered. The rhGTK thus far studied corresponds to a cytosolic form. However, we note from inspection of the human genome that alternative splicing could theoretically generate a 94-amino addition at the N-terminus that contains a mitochondrial-targeting sequence.

### 4.17.7.2 Mitochondrial Aspartate Aminotransferase

AspAT catalyzes the reversible transamination of glutamate and oxaloacetate to $\alpha$-ketoglutarate and aspartate. Almost all mammalian tissues investigated, with the notable exception of red blood cells, contain high levels of cytosolic and mitochondrial isozymes of AspAT. This ubiquity attests to the importance of these enzymes in amino acid metabolism. The two enzymes are crucial components of the malate–aspartate shuttle for the transport of reducing equivalents (in lieu of NADH) between cytosol and mitochondrion (Fitzpatrick et al. 1983). Oxaloacetate and $\alpha$-ketoglutarate link amino acid metabolism to the TCA (tricarboxylic acid) cycle. Coupling of an $\alpha$-ketoglutarate/glutamate-linked aminotransferase to AspAT and the glutamate dehydrogenase reaction permits the flow of nitrogen from amino acid to glutamate and thence to ammonium. When this occurs in the perportal cells of the liver this flow directs excess amino acid nitrogen toward ammonium for urea synthesis. For a review on the metabolic importance of AspAT, see Cooper (1988).

The sequences of many mitAspAT and cytAspAT enzymes are known, including those of human, pig, rat, and chicken. Several AspAT enzymes have been crystallized including chicken mitochondrial and cytosolic forms, the pig cytosolic form, and several bacterial forms (Mehta and Christen 2000; Schneider et al. 2000). They belong to the Fold Type I family, subclass I. It has long been known that the two isozymes of AspAT can utilize amino acids other than glutamate and aspartate as amino acid substrates. For example, they exhibit some activity with the aromatic amino acids (Miller and Litwack 1971; Shrawder and Martinez-Carrion 1972). Rat liver mitAspAT was also shown to readily catalyze transamination of cysteine (Shrawder and Martinez-Carrion 1972). Thus, it occurred to us that this enzyme might be able to utilize cysteine $S$-conjugates as substrates and this turned out to be the case with rat liver mitAspAT (Cooper et al. 2002b; Table 1). To the best of our knowledge the ability of human mitAspAT to catalyze a cysteine $S$-conjugate $\beta$-lyase reaction has not been investigated. However, given the similarity of members of the AspAT family it seems a reasonable assumption that human mitAspAT will catalyze such a reaction.
4.17.8 Role of the Mercapturate Pathway and Cysteine S-Conjugate β-Lyases in the Bioactivation of Toxic Halogenated Alkenes

Acivicin, a selective inhibitor of GGT, protects renal tubule cells against DCVC-induced toxicity (Elfarra et al. 1986; Lash and Anders 1986). On the other hand, it has been reported that acivicin does not protect rats against the nephrotoxicity of hexachloro-1,3-butadiene (Davis 1988). However, others have shown that S-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine (PCBC; the cysteine S-conjugate of hexachloro-1,3-butadiene) is cytotoxic to LLC-PK1 cells (Blackmore et al. 2002). Aminooxyacetate (AOA), a general inhibitor of PLP enzymes protects against DCVC-induced toxicity (e.g., Beuter et al. 1989; Blackmore et al. 2002; Chen et al. 1990, 1994; Lash et al. 1986b) and PCBC-induced toxicity (Blackmore et al. 2002) in LLC-PK1 cells. AOA partially protects against DCVC-induced toxicity in isolated rat kidney proximal tubules (Lash and Anders 1986), but apparently not in isolated human renal proximal tubule cells (Cummings and Lash 2000). α-Keto acids stimulate the toxicity of DCVC toward rat renal cells (Elfarra et al. 1987), suggesting involvement of aminotransferases in the bioactivation process. AOA protects isolated rat proximal tubule cells against TFEC and the cysteine S-conjugates of chlorotrifluoroethylene, 1,1-dichloro-2,2-difluoroethylene, and 1,1-dibromo-2,2-difluoroethylene (Boogaard et al. 1989). α-Methyl DCVC, which cannot be metabolized by a β-lyase reaction, is not toxic (Anders et al. 1988). Taken together, despite an occasional study to the contrary, the literature strongly supports important roles for GGT and cysteine S-conjugate β-lyases in promoting the nephrotoxicity of halogenated alkenes. However, it appears that human renal tissue is less susceptible than that of rat to damage from haloalkene cysteine S-conjugates, presumably as a result of lower specific activities of cysteine S-conjugate β-lyases in human renal tissue (Iyer and Anders 1996; Lash et al. 1990b) and allometric scaling (Anders 2004).

Although the evidence is compelling that cysteine S-conjugate β-lyase reactions contribute to the nephrotoxicity of DCVC and other cysteine S-conjugates derived from halogenated alkenes, as noted above, the sulfoxides of the cysteine S-conjugate and mercapturates may be even more toxic than the cysteine S-conjugate (Lash et al. 1994, 2003; Werner et al. 1996). It has been suggested that the nephrotoxicity of DCVC sulfoxide is related in part to oxidative stress, possibly as a result of depleted mitochondrial GSH stores (Lash et al. 1994, 2003). The β-lyase pathway may result in greater bioactivation of DCVC than does the oxidation pathway in rat proximal tubules, whereas the converse may be true for human proximal tubules (Cummings and Lash 2000).

The cysteine S-conjugate and corresponding mercapturate of both the cis- and the trans-isomers of 1,3-dichloropropene (a soil fumigant) are cytotoxic to renal proximal tubules and in LLC-PK1 cells (Anders 2008; Park et al. 1992). The cytotoxicity is inhibited by methimazole but not by AOA, indicating role for FMO, but not cysteine S-conjugate β-lyases. It was suggested that the cysteine S-conjugate sulfoxide (S-(3-chloroallylsulfinyl)-L-cysteine) undergoes a [2,3]-sigmatropic rearrangement to the sulfinate ester 2-amino-3-(1-chloroallyloxycarbonyl)propanoic acid, which may decompose to toxic acrolein and cysteine sulfinyl chloride (Anders 2008; Park et al. 1992).

Thus, although much of the focus of this section is on bioactivation of haloalkene cysteine S-conjugates via cysteine S-conjugate β-lyases, it is important to note that other bioactivation pathways exist. Moreover, species and sex differences in the mechanisms by which certain haloalkene cysteine S-conjugates are bioactivated must also be considered when evaluating the nephrotoxicity of halogenated cysteine S-conjugates.

4.17.9 Mechanisms Contributing to the Nephrotoxicity of Haloalkene Cysteine S-Conjugates – Toxicant Channeling

Haloalkene cysteine S-conjugates are especially toxic to renal mitochondria (e.g., Anders et al. 1988; Chen et al. 2001; Groves et al. 1993; Lash and Anders 1986). Therefore, it follows that mitochondrial cysteine S-conjugate β-lyases may be particularly important in bioactivating toxic cysteine S-conjugates.

Toxicity of DCVC to kidney cells has been associated with (1) covalent modification of macromolecules, (2) depletion of nonprotein thiols (presumably mostly GSH), and (3) lipid peroxidation (Beuter et al. 1989; Chen et al. 1990; Groves et al. 1991). These effects may be attributable to formation of metabolites derived from a β-lyase reaction since, as mentioned above,
AOA can partially protect against DCVC toxicity in renal tissues and in renal cells in culture. Mechanisms by which metabolites of DCVC and other nephrotoxic haloalkene cysteine S-conjugates alter thiol status may involve direct inhibition of glutathione reductase (Lock and Schnellmann 1990) and generation of oxidative stress that leads indirectly to oxidation of thiol compounds (Chen et al. 1990). Oxidation of DCVC to DCVC sulfoxide followed by formation of a glutathione S-conjugate resulting from the reaction of DCVC sulfoxide with GSH may also lead to oxidative stress (Lash et al. 1994; Sausen and Elfarra 1991). Other studies lead us to suggest an additional possibility, namely that oxidative stress may result in part from Michael addition of thiols to aminoacrylate generated in the β-lyase reaction. In experiments in which LLC-PK1 cells were exposed to DCVC, loss of nonprotein thiols was about 40–60% (Chen et al. 1990). Interestingly, the authors suggested that depletion of nonprotein thiols to this extent was insufficient to kill the cells. As cited earlier, BTC is relatively nontoxic, despite the fact that it is metabolized in vivo via cysteine S-conjugate β-lyases (Elfarra and Hwang 1990). We are unaware of any studies on GSH status in renal cells/tissue resulting from exposure to BTC. Evidently, even if GSH is depleted in renal cells exposed to BTC, this insult may not be sufficient to induce overt toxicity. The implication of the work by Chen et al. (1990) is that in order for a cysteine S-conjugate to exhibit toxicity, it must generate reactive sulfur-containing fragments that covalently add to macromolecules. The toxicity may be enhanced, perhaps synergistically, by oxidative stress and by Michael addition between aminoacrylate and susceptible nucleophiles such as GS− and protein sulfhydryls and/or by modification of coenzyme in susceptible PLP-enzymes.

Cysteine S-conjugate β-lyases are ubiquitous in the body. For example, mitAspAT is present in every cell that contains mitochondria. How then can one explain the unusual susceptibility of kidney to haloalkene cysteine S-conjugates? Many factors are presumably involved (Boogaard et al. 1989), including relative distribution of N-acetyltransferase and aminoacylases, cellular and mitochondrial uptake mechanisms, susceptibility to inactivation of cysteine S-conjugate β-lyases and nearby enzymes, relative Km values, competition from natural amino acid substrates, absolute level of the enzyme, presence of the enzyme in mitochondria, and presence of α-keto substrate to keep the enzyme turning over (if the lyase in question is an aminotransferase). A major contributing factor is likely to be the very large surface area of the renal proximal tubules coupled to the extraordinary high renal vascular perfusion. In humans, the kidneys represent 0.4% of the body weight, but receive 25% of the cardiac output (Pfaller and Gstraunthaler 1998); the cortex receives 95% of the renal blood flow. Despite these factors, haloalkene cysteine S-conjugate-induced toxicity is not necessarily confined to renal tubules. As we have noted above, toxicity may also occur in the liver and occasionally in neural tissue, presumably as a consequence of the widespread occurrence of cysteine S-conjugate β-lyases.

The question can be asked “are there any clues as to which enzymes are responsible for the bioactivation of toxic halogenated cysteine S-conjugates?” Table 1 suggests that GTK is an obvious choice. As noted above, GTK activity is present in cytosolic and mitochondrial fractions of rat kidney. However, most of the activity is in the cytosolic fraction (Cooper and Meister 1981) and most of the β-lyase activity toward DCVC in rat kidney mitochondria could not be assigned to GTK (Abraham et al. 1995). Thus, although GTK probably contributes to the bioactivation of toxic halogenated cysteine S-conjugates in rat kidney, other lyases presumably play an important role.

Although the in vitro specific activity of mitAspAT as a β-lyase toward DCVC and TFEC is somewhat lower than that of GTK (Table 1) the very high level of this enzyme in mitochondria (Parli et al. 1987) suggests that it might play a prominent role in bioactivation of nephrotoxic halogenated cysteine S-conjugates (Cooper et al. 2002b). The importance of mitAspAT as a cysteine S-conjugate β-lyase in the bioactivation of TFEC is underscored by the work of Bruschi and coworkers. Bruschi et al. (1993) showed that six kidney mitochondrial proteins are thioacylated after rats are acutely administered a high dose of TFEC. No thioacylation of cytosolic proteins was detected. The six proteins were identified as HSP60, mitHSP70, mitAspAT, aconitase, the E3k (dihydrolipoamide succinyl transferase) enzyme component of the KGDH, the E3 (dihydrolipoamide dehydrogenase) enzyme component of KGDHC, and the E3 enzyme component of the branched-chain keto acid dehydrogenase complex (BCKADHC) (Bruschi et al. 1993, 1994, 1998; James et al. 2002). Lock and Schnellmann (1990) had previously reported that metabolites of haloalkene cysteine S-conjugates (DCVC, TFEC, PCBC) inhibited dihydrolipoyl dehydrogenase (E3). Our findings that mitAspAT catalyzes a β-lyase reaction with
TFEC in vitro and is syn-catalytically inactivated in the process (Cooper et al. 2002b) is consistent with the in vivo finding that this enzyme in kidney is thioacylated after administration of TFEC to rats. We also showed that a high-Mc β-lyase present in kidney copurifies with HSP70 (Cooper et al. 2001). As cited earlier, the high-Mc β-lyase in rat kidney mitochondria contains mitAspAT. This finding provides a mechanism for the thioacylation of kidney mitHSP70 in rats administered TFEC. Conceivably, mitochondrial HSP60 may also associate with a PLP-containing enzyme that catalyzes a β-lyase reaction with TFEC.

Although the E2k and E3 enzyme components of KGDHC (and the E3 enzyme component of BCKADHC) are thioacylated in kidney mitochondria of rats administered TFEC, the E2p (dihydrolipoamide acetyl transferase) and E3 enzyme components of PDHC are not (Bruschi et al. 1998; James et al. 2002). Moreover, the specific activity of KGDHC, but not that of PDHC, is diminished in the kidneys of TFEC-treated rats (Bruschi et al. 1998). We have found that KGDHC, but not PDHC, is directly inhibited in PC12 cells exposed to 1 mmol l−1 TFEC (Park et al. 1999). These findings are remarkable because E3 is a common component of KGDHC, PDHC, and BCKADHC (and the glyphosate cleavage system). In the presence of TFEC and purified GTK (a source of thioacylating moieties), purified PDHC is more resistant to in vitro inactivation than is purified KGDHC (Park et al. 1999). Moreover, there is some evidence that E3 enzyme components are bound to the complex via E3 enzyme components in PDHC, whereas E3 enzyme components are bound to the complex via E3 enzyme components in KGDHC (McCartney et al. 1998). Thus, part of the resistance of PDHC to thioacylation/inactivation in kidney mitochondria of TFEC-treated rats may be due to differences in the arrangement of its constituent enzymes. In addition, PDHC is not associated with any aminotransferases/β-lyases, whereas KGDHC is closely associated with mitAspAT. Thus, the susceptibility of KGDHC to thioacylation by a fragment derived from TFEC may be due to the proximity of mitAspAT or actual colocalization with subunit enzymes (James et al. 2002; Park et al. 1999).

Several TCA cycle enzymes and its associated enzymes are arranged in supramolecular complexes (metabolons) that facilitate channeling of substrate from one enzyme to another. For example, mitAspAT is part of a metabolon affiliated or localized with KGDHC (see references cited in Cooper et al. 2002a). Based on our findings (Cooper et al. 2002b, 2003) and those of Bruschi and coworkers (Bruschi et al. 1998; James et al. 2002), we have proposed that not only are metabolites channeled through supramolecular complexes in the TCA cycle, but toxicants are also channeled (Cooper et al. 2002a) (Figure 6). This concept explains not only the susceptibility of KGDHC to TFEC-induced inactivation in rat kidney and cells in culture, but also the susceptibility of aconitase and BCKADHC to thioacylation/inactivation. Aconitase is part of a metabolon that includes KGDHC and mitAspAT (James et al. 2002; Ovádi and Srere 2000). Recent evidence also suggests that substrate channeling occurs between a metabolic unit (termed branched-chain amino acid metabolon) consisting of human BCATm and BCKADHC and that the channeling is influenced by the redox state in mitochondria (Islam et al. 2007).

Toxicant channeling may provide an explanation for the finding that the S3 segments of the proximal tubules are especially vulnerable to cysteine S-conjugates derived from halogenated alkenes. The S3 region of the proximal tubule has a high metabolic rate (Korrapat et al. 2007) and KGDHC is an important control step in the TCA cycle (Gibson et al. 2005). Thus, inactivation of KGDHC by chemically reactive compounds released from the β-lyase activity is expected to be especially serious in the S3 segments. Inhibition of KGDHC and aconitase may lead to metabolic stress, which in turn may lead to oxidative stress.

4.17.10 Toxic Homocysteine S-Conjugates

A few studies have been carried out with the homocysteine S-conjugate analogues of DCVC and BTC (Anders et al. 1988; Lash et al. 1986a, 1990a,b). While these compounds are unlikely to be formed from electrophilic xenobiotics in vivo to any large extent they are instructive model compounds. S-(1,2-Dichlorovinyl)-L-homocysteine (DCVHC) is even more toxic than DCVC and the toxicity is potentiated by α-keto acids. Several amino acids that contain a good leaving group in the γ position undergo a non-enzymatic β,γ-elimination reaction when converted to the corresponding amino acid by an aminotransferase (Hollander et al. 1989 and references quoted therein). Activation of the beta C–H bond in the α-keto acid facilitates a
\( \text{XCH}_2\text{CH}_2\text{C(O)CO}_2^- \rightarrow \text{XH} + \text{CH}_2=\text{CHC(O)CO}_2^- \)

(eq 31)

\( \beta,\gamma \)-elimination reaction with the production of vinylglyoxylate (2-oxo-3-butenoic acid) (eqn [31]). This compound is extremely unstable but can be trapped with a suitable mercaptan (Cooper et al. 1976; Lash et al. 1990a).

The increased toxicity of DCVHC relative to DCVC is presumably due to the fact that both compounds generate a toxic reactive sulfur-containing fragment following an elimination reaction. However, the other fragment generated in the \( \beta \)-lyase reaction on DCVC is aminoacrylate, which is presumably less toxic than vinylglyoxylate generated in the \( \beta,\gamma \)-elimination reaction on the keto analogue of DCVHC (Lash et al. 1990a).

### Figure 6
Model of toxicant channeling of \( \beta \)-lyase-derived TFEC products in mitochondria. TFEC is transported into the mitochondrion where it is converted by cysteine S-conjugate \( \beta \)-lyases to aminoacrylate (AMAC) and a reactive sulfur-containing fragment (RSH). AMAC is nonenzymatically converted to pyruvate and ammonium, but also may react with PLP cofactor or susceptible protein residues. The three mitochondrial \( \alpha \)-keto acid dehydrogenase complexes (BCKADHC, KGDHC, PDHC) are represented as multimeric units. Mitochondrial cysteine S-conjugate \( \beta \)-lyases include the homodimeric BCAT\(_m\) and the homodimeric mitAspAT. The close juxtapositioning of BCAT\(_m\) and mitAspAT to enzymes of energy metabolism results in channeling of toxicants to BCKADHC and KGDHC/aconitase (Aco 2), respectively, resulting in their inactivation. PDHC is not known to be associated with any aminotransferase/cysteine S-conjugate \( \beta \)-lyase and is not directly inactivated. The curved arrows represent syncatalytic inactivation of BCAT\(_m\) and mitAspAT by (AMAC) and (RSH). IM, inner mitochondrial membrane; OM, outer mitochondrial membrane; M, matrix. Modified from Cooper, A. J. L.; Pinto, J. T. In Biotechnology: Pharmaceutical Aspects. Advances in Bioactivation Research; Elfarra, A. A., Ed.; Springer: New York, 2008.

### 4.17.11 Electrophilic Xenobiotics Metabolized through the Mercapturate Pathway

#### 4.17.11.1 General Considerations

Many xenobiotics have been tested for their ability to be detoxified by the mercapturate pathway. A detailed summary was published by Chasseaud in 1976. No attempt is made here to update this list. Rather, a summary of the types of organic compounds listed by Chasseaud is provided as representative of electrophilic xenobiotics that generate mercapturates when administered to experimental animals (rabbits and rats have been most extensively tested): halogenated benzenes, halogenated nitrobenzenes, other arylendro compounds, chloro-\( S \)-triazines (herbicides), phenoltetrabromophthalains, aralkyl halides, alkyl and alicyclic...
halides, sulfates and nitro compounds, allyl compounds, alkyl methanesulfonates, organophosphorus compounds, poly cyclic aromatic hydrocarbons (via arene oxides), various α,β-unsaturated compounds (esters, aldehydes, ketones, lactones, nitriles, nitro compounds, sulfones), arylamines, arylhydroxylamines, carbamates, and related compounds. This list exemplifies the versatility and the importance of GSH and the mercapturate pathway for the detoxification of a large number of electrophilic xenobiotics. However, as we have documented above, the pathway may sometimes be a ‘double-edged sword.’ On the one hand, the mercapturate pathway is a defense mechanism to rid the body of potentially noxious xenobiotics. On the other hand, each of the various S-conjugates derived from halogenated alkenes within the mercapturate pathway are toxic as a result of bioactivation of the cysteine S-conjugate by cysteine S-conjugate β-lyases. In the next section we discuss electrophilic drugs that are metabolized by the mercapturate pathway or the mercapturate/β-lyase pathway.

4.17.11.2 Drugs Metabolized by the Mercapturate Pathway or by the Mercapturate/β-Lyase Pathway

Examples of drug electrophiles (discussed by Silverman 1992) that are metabolized in part by pathways involving glutathione S-conjugate formation include the anticancer drug busulfan (see below), the vasodilator nitroglycerin (Needleman et al. 1969) and the immunosuppressive drug azathioprine (de Miranda et al. 1975). Some metabolites of morphine with electrophilic centers are powerful Michael acceptors of GSH (Correia et al. 1984).

The carbonic anhydrase inhibitor methazolamide is metabolized to glutathione S- and a cysteine S-conjugate. The latter is a substrate of cysteine S-conjugate β-lyase(s) in bovine kidney and liver homogenates (Kishida et al. 2001). Cysteine S-conjugate β-lyases are also presumably present in eye tissues. Therefore, the β-elimination reaction may account for the binding of a metabolite of methazolamide to macromolecules and for the specific ocular toxicity (Kishida et al. 2001).

Cisplatin is used to treat germ cell tumors, ovarian cancer, head and neck tumors and as a radiation sensitizer for cervical cancer. Unfortunately, its effectiveness can be limited particularly during tumor recurrence by its toxicity to renal proximal tubule cells (reviewed in Zhang and Hanigan 2003). DNA damage is the primary mechanism by which cisplatin kills tumor and other dividing cells. However, the renal proximal tubule cells are well-differentiated, nondividing cells that are not killed by other DNA-damaging agents (Hanigan and Devarajan 2003). Evidence has been presented that damage to kidney cells is due to conversion of cisplatin to its glutathione S-conjugate and subsequently to its cysteine S-conjugate. The cysteine S-conjugate is then bioactivated by cysteine S-conjugate β-lyase(s) to generate a fragment containing a Pt-SH moiety. This Pt-SH fragment is proposed to react with macromolecules at thiophilic centers (Zhang and Hanigan 2003; Figure 7).

After mice were treated with cisplatin, proteins in kidney mitochondria were more platinated than proteins in the cytosolic fraction (Zhang et al. 2006). Moreover, the platination was decreased in the mitochondrial fraction, but not in the cytosolic fraction, in mice pretreated with AOA. The specific activities of mitAspAT, aconitase, and especially KGDHC were decreased in LLC-PK1 cells treated with cisplatin (Zhang et al. 2006). The specific activity of KGDHC was decreased even further in cisplatin-treated LLC-PK1 cells overexpressing mitAspAT. The data are consistent with the hypothesis that the cisplatin cysteine S-conjugate is a β-lyase substrate of mitAspAT and that the released Pt-SH fragment reacts with proteins in kidney mitochondria especially KGDHC.

Another interesting example of a drug that is metabolized through the mercapturate pathway is busulfan (Marchand et al. 1988). Busulfan is a bifunctional alkylating agent used for the treatment of hematological and other malignancies prior to stem cell transplantation (e.g., Iwamoto et al. 2004). The mercapturate pathway of busulfan metabolism was shown to occur in rats by the detection of the sulfonium mercapturate, N-acetyl-β-(S-tetrahydrothiophenium)-l-alanine in the urine (Hassan and Ehrrson 1987). Busulfan is converted to a glutathione S-conjugate (l-γ-glutamyl-l-β-(S-tetrahydrothiophenium)-l-alanylglcyline) by direct interaction with GSH (Ritter et al. 1999) and enzymatic catalysis by GSTs, especially GSTA1-1 (Czerwinska et al. 1996; Gibbs et al. 1996; Ritter et al. 1999, 2002). Oxidation products of tetrahydrothiophene (THT) make up the majority of identified busulfan metabolites in rodents and rabbits (Roberts and Warwick 1961). 1,4-Diiodobutane and 1,4-dibromobutane are also converted to 1-γ-glutamyl-β-(S-tetrahydrothiophenium)-l-alanylglcyline and to mercapturate in rat (Marchand and Abdel-MoneM 1985; Marchand et al. 1988; Onkenhout et al. 1986).
The busulfan-glutathione adduct undergoes a nonenzymatic $\beta$-elimination reaction yielding THT at pH 8.0 (Roberts and Warwick 1961). Presumably, therefore, THT can arise nonenzymatically from the glutathione $S$-conjugate in vivo. However, THT is also likely to arise via an enzymatic route (Cooper et al. 2008b). The detection of mercapturate in rat shows that the cysteine $S$-conjugate must have been generated in vivo either by direct reaction of busulfan with cysteine and/or via the glutathione $S$-conjugate. The cysteine $S$-conjugate of busulfan ($\beta$-(S-tetrahydrothiophenium)-L-alanine; THT-A) is unusual in that the sulfur is in the oxidation state of a sulfonium rather than a thioether. Since sulfonium compounds are high-energy compounds a $\beta$-elimination reaction yielding THT and aminoacrylate should be energetically favorable. Indeed we have found that (1) homogenates of rat liver, kidney, and brain, (2) $\mu$mol 1$^{-1}$ PLP, and (3) highly purified cystathionine $\gamma$-lyase can catalyze a $\beta$-elimination reaction with THT-A (Cooper et al. 2008b). A scheme for the possible routes involved in busulfan metabolism is shown in Figure 8.

It is not clear whether the $\beta$-elimination reactions on balance are detoxication or bioactivation events. Formation of THT is probably a detoxication event. However, the formation of aminoacrylate may be a bioactivation event if it leads to selected enzyme inactivation or removal of thiols. The formation of a GSH analogue in which the cysteine of GSH is replaced by a dehydroalanine residue ($\gamma$-glutamyldehydroalanylglycine; Figure 8, structure X) may also be a bioactivation event. It is expected that Michael addition of sulfides across the double will occur. Addition of cysteine or GSH will give rise to peptides bridged by lanthionine residues. Such adduct formation might elicit a toxic response by lowering GSH levels or by producing GSH analogues that interfere with normal GSH functions.

### 4.17.12 Conclusions

Conversion of an electrophile to the corresponding glutathione $S$-conjugate and thence to the mercapturate followed by excretion represents a detoxication process. A very large number of potentially toxic electrophilic xenobiotics are eliminated by this process. In addition, some natural metabolites (e.g., leukotrienes, prostaglandin (PG)$A_2$, 15-deoxy-{$\Delta^{12,14}$}-PGJ$_2$, hydroxynonenal) may be eliminated as their mercapturates. However, formation of a glutathione $S$-conjugate may sometimes lead to bioactivation. For example, glutathione $S$-conjugates of some hydroquinones and dihaloethanes may be directly toxic. In other cases, the cysteine
Figure 8 Proposed mechanism for the metabolic conversion of busulfan to THT. The strong electron-withdrawing properties of the –OS(O2)CH3 group on busulfan (I) results in electron deficiency at each carbon adjacent to the two methanesulfonyl groups. This deficiency facilitates the nucleophilic attack of the sulfhydryl of GSH at one of these carbons, resulting in elimination of methanesulfonate (II) and subsequent generation of adduct III. This step occurs nonenzymatically but is accelerated by GSTs. The electron deficiency in the remaining carbon adjacent to the –OS(O2)CH3 group in adduct III facilitates nonenzymatic intramolecular nucleophilic attack at this carbon by a pair of electrons on the sulfur of the thioether, generating the glutathione S-conjugate (V) and eliminating a second equivalent of methanesulfonate. The conjugate (V) possesses a cyclic sulfonium group. GGT (a) and dipeptidases (b) possess broad specificities. Thus, V is expected to be readily converted to the corresponding cysteine S-conjugate THT-A (VI). VI may also be formed by nonenzymatic nucleophilic attack of a cysteine sulfur on busulfan (I), which generates adduct IV and methanesulfonate, followed by intramolecular cyclization and elimination of a second equivalent of methanesulfonate. VI contains a strong electron-withdrawing group and is therefore expected to undergo a facile β-elimination reaction to yield THT (VII), ammonium (VIII), and pyruvate (IX). This reaction occurs nonenzymatically at pH 7.4, but is accelerated by PLP and by rat liver cystathionine γ-lyase (and by other as yet unrecognized enzymes in rat tissues). THT (VII) may also be generated directly and nonenzymatically from γ-E-THT-AG (V) by an elimination reaction that results in the formation of γ-glutamyldehydroalanylglycine (X). The conversion of busulfan (I) to the conjugate (V) is shown as occurring in two consecutive steps, but may occur by a concerted mechanism. Note that the sulfur in THT is not derived from busulfan, but rather from the sulfur of GSH.
S-conjugate derived from the glutathione S-conjugate may be toxic. For example, the cysteine S-conjugate of dopamine can form potentially highly toxic benzothiazines. In other cases, the sulfur of the thioether linkages of the S-conjugates of the mercapturate pathway may be oxidized to sulfoxides that are more toxic than the corresponding thioethers. For example, DCVC sulfide is more toxic than the corresponding thioether. The potential exists for the cysteine S-conjugate sulfoxide to undergo an elimination reaction to generate a reactive sulfenic acid. However, there is no evidence that this occurs in the case of DCVC sulfoxide. In this case, toxicity may be associated with depletion of GSH.

If the cysteine S-conjugate formed in the mercapturate pathway contains a good electron-withdrawing group (good nucleofuge), it may undergo a β-elimination reaction. This reaction is catalyzed by several PLP-containing cysteine S-conjugate β-lyases that are normally involved in amino acid metabolism. If the eliminated sulfur-containing fragment is not especially reactive the parent cysteine S-conjugate may not be particularly toxic. The sulfur-containing fragment may be thiomethylated or S-glucuronidated and excreted. Alternatively, the fragment may be further oxidized to a sulfoxide or sulfone before excretion. On the other hand, if the eliminated sulfur-containing fragment is chemically reactive (e.g., by covalently reacting with macromolecules) the parent cysteine S-conjugate may be toxic, especially to the kidneys. Electrophiles that are bioactivated by this mechanism include halogenated alkenes and drugs such as methazolamide and cispłatim. Some PCBS and amiphenols may also be metabolized in part by pathways involving cysteine S-conjugate β-lyases. However, the contribution of the β-lyase reaction to the toxicity of these compounds is not clear. Mitochondrial enzymes of energy metabolism (KGDHC, aconitase) are especially vulnerable to reactive fragments generated from toxic halogenated, cysteine S-conjugates, by a process that has been termed toxicant channeling. Humans are exposed to a large number of exogenously and endogenously produced electrophiles. It is, therefore, possible that cysteine S-conjugate β-lyases contribute to mitochondrial dysfunction of aging and disease.

The recent discovery that the cysteine S-conjugate of busulfan/dihalobutane, which is a sulphonium conjugate, can undergo enzyme-catalyzed β-elimination suggests that other drugs or xenobiotics may undergo similar transformations involving sulphonium conjugates. 1-(Glutathione-S-yl)thiiranium and the corresponding cysteine S-conjugate (1-(2-amino-2-carboxyethyl)thiiranium) formed from dihalopropanes are directly highly toxic. By analogy with the cysteine S-conjugate β-lyase reaction with the cysteine S-conjugate THT-A it may be possible that a β-lyase reaction on 1-(2-amino-2-carboxyethyl)thiiranium will lead to elimination of thirane, which may possibly be a bioactivation event.

In view of (1) the large number of mammalian cysteine S-conjugate β-lyases identified to date, (2) their overlapping specificities, (3) their widespread occurrence in tissues, (4) their presence in different subcellular compartments (e.g., cytosol, mitochondria, and peroxisomes), and (5) the increasingly recognized wide scope of these reactions, the potential of these enzymes for generating toxic products in different subcellular compartments, not only in the kidneys but also in other tissues, is highly significant. In addition, it is becoming apparent that some plants and microorganisms use the mercapturate pathway to generate useful sulfur-containing compounds. We suggest that metabolism of some natural products, certain drugs, and endogenously produced electrophiles via pathways that include the mercapturate pathway coupled to cysteine S-conjugate β-lyases may be more common than is generally appreciated.

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