



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

10.17 Metabolism of Glutathione S-Conjugates: Multiple Pathways

AJL Cooper, New York Medical College, Valhalla, NY, United States

MH Hanigan, University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States

© 2018 Elsevier Ltd. All rights reserved.

10.17.1	Introduction	365
10.17.2	Enzymes of the Mercapturate Pathway	367
10.17.2.1	Glutathione S-Transferases (GSTs)	367
10.17.2.2	γ -Glutamyltransferases	368
10.17.2.2.1	GGT family of enzymes	368
10.17.2.2.2	Protein structure	368
10.17.2.2.3	Expression of GGT	369
10.17.2.2.4	Localization of GGT	369
10.17.2.2.5	Reactions catalyzed by GGT	370
10.17.2.2.6	Inhibitors of GGT	370
10.17.2.2.7	GGT and disease	370
10.17.2.2.8	γ -Glutamyl leukotrienase	370
10.17.2.3	Hydrolysis of Cysteinylglycine and Its Conjugates	371
10.17.2.3.1	Aminopeptidase N (APN)	371
10.17.2.3.2	Cysteinylglycine dipeptidase	372
10.17.2.3.3	Leucyl aminopeptidase	372
10.17.2.4	Cysteine S-Conjugate N-Acetyltransferases	372
10.17.3	Interorgan Transport of Mercapturates and Mercapturate Intermediates	372
10.17.4	Biotransformations of Endogenous Compounds through the Mercapturate Pathway	375
10.17.4.1	Deactivation/Detoxification	375
10.17.4.2	Bioactivation	376
10.17.5	Metabolic Pathways Diverging From Mercapturate Biosynthesis	376
10.17.5.1	Deconjugation	376
10.17.5.2	Deconjugation Coupled to Reductive Dehalogenation	377
10.17.5.3	Hydrolytic Deconjugation	377
10.17.5.4	Bis(glutathionyl) Conjugate Formation	378
10.17.5.5	Deamination and Transamination	379
10.17.5.6	Sulfur Oxidation of Components of the Mercapturate Pathway	379
10.17.5.7	Thiomethyl Shunt	379
10.17.5.8	Aminoacylase-Catalyzed Deacetylation of Mercapturic Acids	380
10.17.6	Cysteine S-Conjugate β-Lyases	380
10.17.6.1	Background: Bioactivation of Halogenated Alkenes	380
10.17.6.2	Possible Repair Mechanisms	381
10.17.6.3	The Cysteine S-Conjugate β -Lyase Reaction	381
10.17.6.4	Cysteine S-Conjugate β -Lyases in Enteric Bacteria	382
10.17.6.5	Cysteine S-Conjugate β -Lyases in Mammals	383
10.17.6.6	Cysteine S-Conjugate β -Lyases in Other Organisms	383
10.17.6.7	Reactive Sulfur-Containing Fragments Generated by β -Lyase Reactions Involving Halogenated Alkene Cysteine S-Conjugates	384
10.17.6.8	Catalytic Mechanism of the Cysteine S-Conjugate β -Lyase Reaction and Syncatalytic Inactivation	385
10.17.7	Major Cysteine S-Conjugate β-Lyases of Mammalian Tissues	387
10.17.7.1	GTK/Kynurenine Aminotransferase I	387
10.17.7.2	Mitochondrial Aspartate Aminotransferase	388
10.17.8	Role of the Mercapturate Pathway and Cysteine S-Conjugate β-Lyases in the Bioactivation of Toxic Halogenated Alkenes	388
10.17.9	Mechanisms Contributing to the Nephrotoxicity of Haloalkene Cysteine S-Conjugates: Toxicant Channeling	389
10.17.10	Homocysteine S-Conjugates	391
10.17.11	Electrophilic Xenobiotics Metabolized through the Mercapturate Pathway	391
10.17.11.1	General Considerations	391
10.17.11.2	Drugs Metabolized by the Mercapturate Pathway or by the Mercapturate/ β -Lyase Pathway	391
10.17.12	Conclusions	394

This is an update of Cooper, A.J.L., Hanigan, M.H., Enzymes Involved in Processing Glutathione Conjugates, Comprehensive Toxicology, Second Edition, edited by Charlene A. McQueen, Elsevier, Oxford, 2010, Volume 4, Pages 323–366.

Acknowledgments	394
References	395
Relevant Websites	406

Glossary

Aminoacylase An enzyme that catalyzes the deacylation of *N*-acetyl-L-amino acids including mercapturates.

Bioactivation A process by which a compound is converted to a metabolite that is more toxic than the original compound—also known as toxification.

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.

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 ion, and a sulfur-containing fragment.

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 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 a β -lyase with many selenocysteine Se-conjugates.

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 and/or bile.

Mitochondrial aspartate aminotransferase A very important enzyme in amino acid metabolism and present in high amounts in all cells 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.

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-conjugate, cysteine S-conjugates, and *N*-acetyl cysteine S-conjugate.

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 β -lyase pathway followed by an enzyme-catalyzed S-methylation reaction with S-adenosyl-L-methionine as methyl donor. The resulting thiomethyl compound may be excreted unchanged or following oxidation to a sulfoxide/sulfone.

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. The process may be of wider applicability if cysteine S-conjugate β -lyases (other than aspartate aminotransferase) are present in tight metabolic linkages with other enzymes.

γ -Glutamyl leukotrienase A unique enzyme of the γ -glutamyltransferase family that metabolizes 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 products of the reaction is glutamate. However, at exceptionally high, nonphysiological concentrations, amino acids or dipeptides may be γ -glutamyl acceptors, generating a γ -glutamyl amino acid or a γ -glutamyl dipeptide, respectively. The enzyme is also referred to as γ -glutamyl transpeptidase and GGT1.

Abbreviations

AGAT II	Alanine-glyoxylate aminotransferase isoenzyme II
AlaAT	Alanine aminotransferase
ANIT	1-Naphthylisothiocyanate
AOA	Aminooxyacetate
APN	Aminopeptidase N
AspAT	Aspartate aminotransferase
BCAT _c	Cytosolic branched-chain amino acid aminotransferase
BCAT _m	Mitochondrial branched-chain amino acid aminotransferase
BCKADHC	Branched-chain keto acid dehydrogenase complex
BTC	S-(2-Benzothiazolyl)-L-cysteine
CCBL1	Cysteine conjugate β-lyase 1
CCBL2	Cysteine conjugate β-lyase 2
Cyt	Cytosolic
DCVC	S-(1,2-Dichlorovinyl)-L-cysteine
DCVHC	S-(1,2-Dichlorovinyl)-L-homocysteine
FMO	Flavin-dependent monooxygenase
GGT	γ-Glutamyltransferase
GSH	Glutathione (γ-glutamylcysteinylglycine)
GST	Glutathione S-transferase
GTK	Glutamine transaminase K
GTL	Glutamine transaminase L
KAT	Kynurenine aminotransferase
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
TCVC	S-(1,2,2-Trichlorovinyl)-L-cysteine
TFEC	S-(1,1,2,2-Tetrafluoroethyl)-L-cysteine
THT	Tetrahydrothiophene
THT-A	β-(S-Tetrahydrothiophenium)-L-alanine
UTR	Untranslated region

10.17.1 Introduction

Mercapturic acids (S-(N-acetyl)-L-cysteine conjugates) were first identified over 135 years ago and were soon recognized to play a role in the detoxification 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 cysteinyl 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 mmol l⁻¹ (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 various peptidases. Finally, the cysteine S-conjugate is N-acetylated to the corresponding N-acetyl-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 Fig. 1.

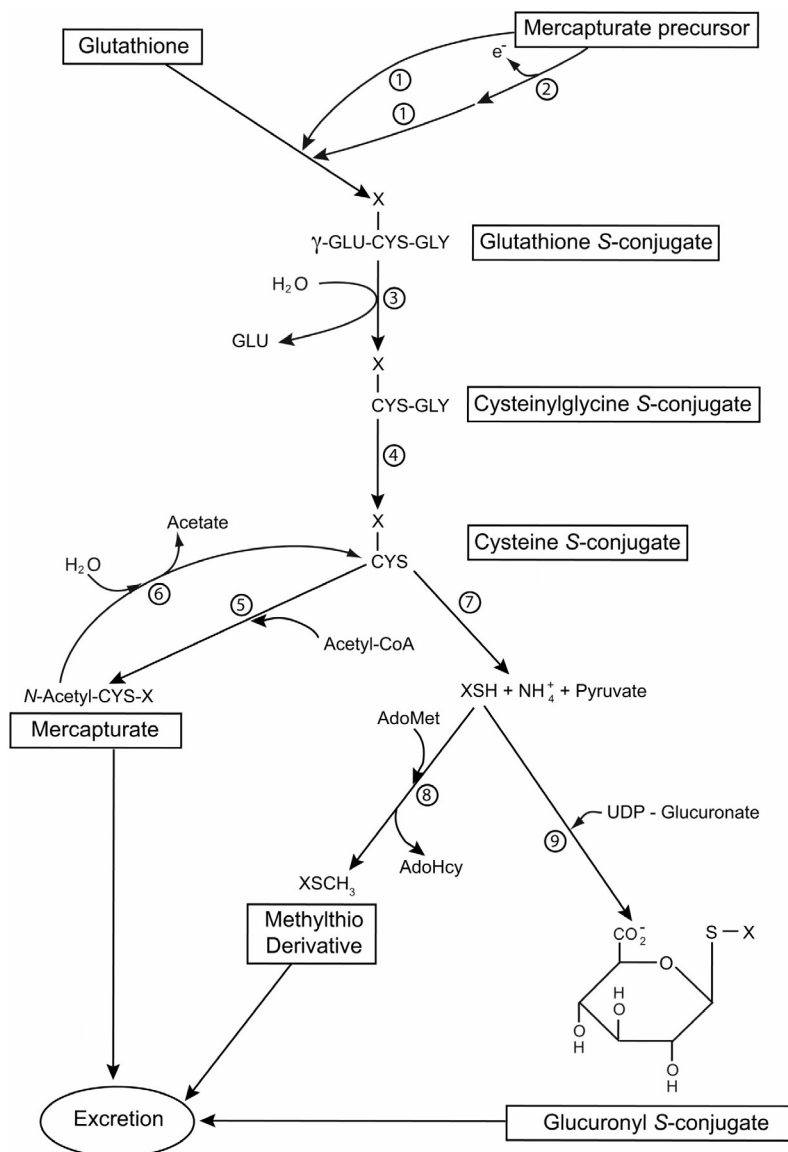


Fig. 1 The mercapturate pathway and associated side reactions. If the mercapturate precursor contains an electrophilic center, it may react directly with GSH (reaction 1). Alternatively, the precursor may be converted to a compound with an electrophilic center (reaction 2) prior to reaction with GSH. Reactions (1)–(5) represent the mercapturate pathway. Reactions (7)–(9) are alternative reactions for elimination of cysteine S-conjugates. Reactions (7) plus (8) denote the thiomethyl shunt. The thiomethyl compound (XSCH_3) may be excreted unchanged or further oxidized to sulfoxide, sulfone, or CO_2 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 “**Deamination and Transamination**” section). The cysteine S-conjugate may also be converted to the corresponding sulfoxide. Enzymes involved: (1) glutathione S-transferases (GSTs), (2) oxidases that generate an electrophilic center for attack by GSH (in some cases oxidation may be nonenzymatic), (3) ectoenzyme γ -glutamyltransferase (GGT), (4) ectoenzymes APN/cysteinylglycine dipeptidase and possibly cytosolic leucyl aminopeptidase, (5) *N*-acetyltransferases, (6) aminoacylases, (7) cysteine S-conjugate β -lyases, (8) thio-methyltransferase, and (9) UDP-glucuronosyltransferases. Many potentially toxic xenobiotics and a few endogenous compounds are metabolized through the mercapturate pathway. *AdoHcy*, *S*-adenosyl-L-homocysteine and *AdoMet*, *S*-adenosyl-L-methionine. Modified from Silbernagl, S., Heuner, A., 1993. In Anders, M.W., Dekant, W., Henschler, D., Oberleithner, H., Silbernagl, S. (eds.), *Renal Disposition and Nephrotoxicity of Xenobiotics*. Academic Press, Inc., San Diego, CA. pp. 135–154. Cooper, A.J.L., Pinto, J.T., 2008. In Elfarra, A.A. (ed.), *Biotechnology: Pharmaceutical Aspects*. Advances in Bioactivation Research. Springer, New York, NY.

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 detoxification 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 serves as 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 ("γ-Glutamyl leukotriene-nase" and "Deactivation/Detoxification" sections). However, in other cases, a diversion of the pathway leads to the bioactivation of xenobiotics rather than to a detoxification. Thus, cysteine S-conjugates formed from some halogenated alkenes are converted to pyruvate, ammonium ion, and a reactive, toxic fragment by the action of cysteine S-conjugate β-lyases ("Background: Bioactivation of Halogenated Alkenes" section). Leukotriene transformations and cysteine S-conjugate β-lyases are considered in more detail in the succeeding text.

The purpose of this article 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 article also deals at some length with nonenzymatic and enzymatic processes that divert glutathione S-conjugate and cysteine S-conjugate away from the mercapturate pathway.

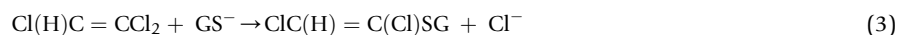
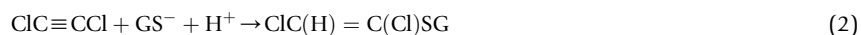
Some of the points discussed in this article have been adapted from reviews by Dekant et al. (1994), Anders (2008), Cooper and Pinto (2008), and Cooper et al. (2011).

10.17.2 Enzymes of the Mercapturate Pathway

10.17.2.1 Glutathione S-Transferases (GSTs)

GSH has many biological roles, including (1) defense against reactive oxygen species and free radicals, (2) defense against reactive electrophiles, though the action of GSTs (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). Recently, it has become apparent that GSTs have important roles, for example, in catalyzing addition of GS⁻ to cyst(e)ine residues resulting in protein glutathionylation (Menon and Board, 2013; Townsend et al., 2014) and in the regulation of mitogen-activated protein kinases (Tew and Townsend, 2012). Many drugs and other xenobiotics or their metabolites are directly electrophilic and are thus capable of causing cellular damage 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 and 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). Thus, reactions of GSH with electrophiles often proceed at a measurable rate in the absence of a catalyst (Koo and Dekant, 1991), but in general, the reaction is 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 monomer *M_r* of ~25 × 10³. Many GST isozymes 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 the nucleophile but, depending on the isozyme, 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 (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 (S_NV) reactions that may be either a Michael addition reaction (e.g., with tetrafluoroethylene (F₂C=CF₂, Eq. 1) and dichloroacetylene (ClC≡CCl, Eq. 2)) or an addition–elimination reaction (e.g., with trichloroethylene (Cl(H)C=CCl₂, Eq. 3)). GSTs also catalyze reactions with epoxide moieties (e.g., in the conversion of leukotriene A₄ (LTA₄) to leukotriene C₄ (LTC₄)). 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).



Interestingly, despite their generally broad specificity, many mammalian GSTs are able to catalyze stereoselective addition of GSH to some electrophiles (Balogh et al., 2008; Dohn et al., 1985; Livesey et al., 1982; Mangold and Abdel-Monem, 1980; Rosner et al., 1998). Some bacterial GSTs also exhibit stereoselectivity (Gall et al., 2014).

10.17.2.2 γ -Glutamyltransferases

10.17.2.2.1 GGT family of enzymes

In 1959, it was postulated that a glutathionase (an enzyme that degrades GSH) plays a role in the formation of mercapturic acids (Bray et al., 1959). Previously, Binkley and Nakamura (1948) had demonstrated that the first step in the metabolism of GSH is cleavage of the γ -glutamyl bond followed by cleavage of the cysteinylglycine peptide bond. The enzyme that cleaves the γ -glutamyl bond of GSH and glutathione S-conjugates was subsequently isolated and named γ -glutamyltransferase (GGT, also known as γ -glutamyltranspeptidase; Orłowski and Meister, 1965; Szewczuk and Baranowski, 1963). A second enzyme that cleaves γ -glutamyl bonds (converting the glutathione S-conjugate LTC₄ to the corresponding cysteinyl-glycine conjugate LTD₄) was identified independently by two groups in the 1990s and named GGT-rel and γ -glutamyl leukotrienase (Carter et al., 1997; Heisterkamp et al., 1991).

In humans, the genes that encode GGT and γ -glutamyl leukotrienase 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). Recent studies of a gene named GGT2, which is present only in humans and has 97% nucleotide identity and 94% amino acid identity with human GGT, have shown that this gene does not encode a functional protein (West et al., 2013a). GGT2 mRNA is expressed, and the protein is synthesized, but the protein does not autocleave into an active enzyme and is rapidly degraded (West et al., 2013a). To date, only GGT and γ -glutamyl leukotrienase have been shown to be expressed as functional proteins.

Heisterkamp and colleagues have collaborated with the Human Genome Organization (HUGO) Gene Nomenclature Committee to standardize the nomenclature for the GGT gene family (Heisterkamp et al., 2008). This nomenclature designates γ -glutamyltransferase as GGT1 and γ -glutamyl leukotrienase as GGT5. For clarity, throughout this article, we will continue to use the descriptive names γ -glutamyltransferase (GGT) and γ -glutamyl leukotrienase. Homologues of GGT are expressed throughout the plant and animal kingdoms (Martin et al., 2007; Morrow et al., 2007; Ubiyovk et al., 2006; Law et al., 2012; Rossi et al., 2012; Verma et al., 2015).

10.17.2.2.2 Protein structure

GGT is a type II membrane protein with a single transmembrane domain within the large subunit (Coloma and Pitot, 1986; Goodspeed et al., 1989; Rajpert-De Meyts et al., 1988; Sakamuro 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 Fig. 2). All of the enzymatic activity takes place in the extracellular portion of the enzyme

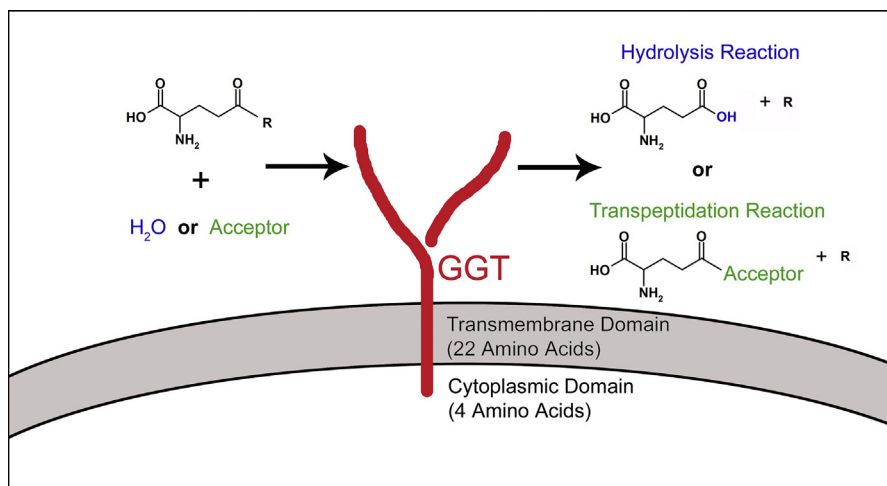


Fig. 2 Diagrammatic structure of γ -glutamyltransferase (GGT). The enzyme is composed of two noncovalently attached subunits. The larger subunit traverses the cellular membrane such that most of the protein is exposed to the extracellular milieu. The smaller subunit is entirely within the extracellular milieu. As a result of its location, GGT is often referred to as an ectoenzyme. GGT catalyzes the hydrolysis of the γ -glutamyl compound to free glutamate and R. At high pH and in the presence of millimolar concentrations of some dipeptides, amino acids, or GSH (as acceptor molecules), the enzyme can catalyze a transpeptidation reaction to generate a γ -glutamyl acceptor and R. Under physiological conditions, however, the GGT reaction is a hydrolysis reaction.

(Horiuchi et al., 1978; Tsao and Curthoys, 1980). The enzyme is translated as a single polypeptide chain and autocleaves into two subunits that remain noncovalently bound to each other (Kinlough et al., 2005; Nash and Tate, 1982; West and Hanigan, 2010). Both subunits are required for activity (Gardell and Tate, 1981). The human enzyme contains seven potential *N*-glycan sites. *N*-Linked glycans have been isolated from GGT and characterized (Yamashita et al., 1983a,b, 1986; West et al., 2010).

A detailed study of the *N*-glycosylation of GGT from the human liver and kidney showed tissue-specific and site-specific *N*-glycosylation of GGT in normal human tissues (West et al., 2010). Rat GGT has been shown to contain two *O*-linked glycans, but no *O*-linked glycans have been reported for human GGT (Blochberger et al., 1989). Cotranslational *N*-glycosylation is required for proper folding and autocleavage of the human GGT propeptide into two subunits, although the *N*-glycans can be removed from the mature enzyme without altering its stability or catalytic activity (West et al., 2011). In addition, a CX3C motif within GGT is also required for proper folding and autocleavage (West et al., 2013a). Treatment 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., 1995a,b). The crystal structure of human GGT has recently been solved (West et al., 2013b). The crystal structures of GGT homologues from *Escherichia coli*, *Helicobacter pylori*, and *Bacillus subtilis* were reported previously (Morrow et al., 2007; Okada et al., 2006; Wada et al., 2010), but the structure of the human enzyme is the first report for any eukaryotic GGT. The crystal structure of the human enzyme reveals a substrate channel with Thr-381, the catalytic nucleophile, in the deepest part of the cleft (West et al., 2013b). Many other insights into the enzyme were gained from an analysis of the structure, including the presence of a bimodal switch in the orientation of the catalytic nucleophile and the open conformation of the lid loop region of the enzyme (West et al., 2013b).

10.17.2.2.3 Expression of GGT

In the rat, GGT is a single copy gene, and its expression is regulated by five tandemly arranged promoters that give rise to seven different transcripts, all of which encode the same protein but have distinct 5'-untranslated regions (UTRs) (Chikhi et al., 1999). The promoters are tissue-specific, and AP-1, AP-2, and nuclear factor κ B (NF κ B) binding sites have been identified in the promoter region (Chikhi et al., 1999). In the mouse, GGT is a single copy gene that can be transcribed from seven different promoters, which give rise to distinct mRNAs that encode the same protein but differ in their 5'-UTR (Lieberman et al., 1995). Induction of GGT is tissue-specific, and murine GGT mRNA has been shown to undergo tissue-specific and developmentally regulated alternative splicing, which gives rise to both active and enzymatically inactive GGT proteins (Chikhi et al., 1999; Joyce-Brady et al., 2001). Binding sites for AP-1 and the redox-sensitive transcription factors AP-2 and NF κ B have been identified in the murine promoters (Chikhi et al., 1999). Expression of *K-ras* and *Myc* oncogenes has been shown to increase the transcription of *Nrf2* and induce a series of genes including GGT, all of which are important in the cellular response to ROS (DeNicola et al., 2011). In *Nrf2*-deficient cells, GGT mRNA is reduced and is not altered by expression of the oncogenic *K-ras*, indicating that *K-ras* is signaling through *Nrf2* to induce GGT mRNA. This was confirmed in *Nrf2* knockdown studies.

The transcriptional regulation of GGT in humans is tissue-specific with multiple mRNAs encoding the same open reading frame but differing in their 5'-UTR (Visvikis et al., 2001; Daubeuf et al., 2001). The human GGT promoter contains binding sites for AP-1, AP-2, CREB, GRE, and NF κ B and two binding sites for Sp1 (Visvikis et al., 2001; Reuter et al., 2009). The 5'-UTR of human GGT also contains multiple steroid modulatory elements (Diederich et al., 1993). Tumor necrosis factor alpha (TNF α) was shown to induce GGT expression through the NF κ B signaling pathway in the human chronic myelogenous leukemia cell lines K562 and MEG-01 (Reuter et al., 2009).

Five distinct mRNAs that encode GGT have been identified in humans and six in mice, and multiple mRNAs have also been identified in rats and pigs (Chikhi et al., 1999; Daubeuf et al., 2001; Visvikis et al., 2001). In each species, the mRNAs all encode the same protein but differ in their UTR. The sequence of the UTR reveals use of multiple promoters that are regulated in a tissue-specific manner. In vertebrates, GGT is constitutively expressed at a high level on the apical surface of the renal proximal tubules, the site of formation of mercapturates from glutathione *S*-conjugates in the kidney (Hanigan and Frierson, 1996; Law et al., 2012; Yamashita et al., 1983a,b). In some tissues, GGT can be induced. The promoters contain binding sites for *Nrf2*, AP-1, AP-2, and NF κ B transcription factors, and induction of GGT under conditions of redox stress has been demonstrated in several species (Reuter et al., 2009; Joyce-Brady et al., 2001; DeNicola et al., 2011; Hanigan, 2014). In the mouse, alternative splicing of the mRNA has been shown to produce at least three GGT isoforms (Chikhi et al., 1999; DeNicola et al., 2011). One of the splicing events is developmentally regulated (Joyce-Brady et al., 2001). In humans, the high level of nucleotide sequence homology between human GGT and GGT2 has resulted in a lack of specificity among some of the primers designed for GGT, which is a confounding variable in many microarray studies of GGT expression in human tissues (West et al., 2013a).

10.17.2.2.4 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 expresses GGT. GSH and glutathione *S*-conjugates present in fluids that flow through these ducts are metabolized by GGT.

10.17.2.2.5 Reactions catalyzed by GGT

GGT catalyzes the cleavage of γ -glutamyl bonds (Fig. 2). A large number of γ -glutamyl compounds in which the glutamate moiety is unrestricted except for the γ -glutamyl bond are substrates for GGT. These include GSH, GSSG, glutathione S-conjugates, γ -glutamyl amino acids, and γ -glutamyl *p*-nitroanilide (a substrate commonly used to assay enzyme activity) (Orlowski and Meister, 1963, 1970; Wickham et al., 2011). The γ -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). The crystal structure of the human enzyme cocrystallized with glutamate showed that the α -carboxylate of the γ -glutamyl moiety of the substrate is stabilized within the active site of the enzyme by forming hydrogen bonds with Arg-107, Ser 451, and Ser-452S (West et al., 2013b). The α -amino group forms a salt bridge with Glu-420. Cleavage of the γ -glutamyl bond of the substrate, the first step in the reaction, results in the formation of an acyl bond between the glutamate and the catalytic threonine of the enzyme with the release of all but the glutamate portion of the substrate. The glutamate is released from the enzyme when the enzyme/ γ -glutamyl complex reacts either with a water molecule, yielding glutamate and free enzyme, or with the α -amine of an acceptor molecule (transferase or transpeptidase reaction), yielding a γ -glutamyl amino acid and free enzyme (Taniguchi and Ikeda, 1998). The α -amine of a free amino acid or a dipeptide can serve as an acceptor for the γ -glutamyl group (Thompson and Meister, 1977). The enzyme is routinely assayed by the development of a yellow color upon the release of *p*-nitroaniline from γ -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 (Thompson and Meister, 1977). 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 GGT reaction is a hydrolysis reaction 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 enables 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 γ -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 (“Hydrolysis of Cysteinylglycine and Its Conjugates” section). 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. A novel assay that measures the GGT hydrolysis reaction at physiological pH and osmolarity has shown that the second order rate kinetics for GSH and glutathione S-conjugates are similar (Wickham et al., 2011).

10.17.2.2.6 Inhibitors of GGT

Glutamine analogs including acivicin, 6-diazo-5-oxo-L-norleucine, and L-azaserine inhibit GGT (Tate and Meister, 1978). However, these glutamine analogs are neurotoxic and cannot be used clinically (Ahluwalia et al., 1990; Hidalgo et al., 1998). γ -Phosphono diester analogs of glutamate have also been shown to be potent inhibitors of GGT (Han et al., 2007; Nakajima et al., 2014). S-Alkyl L-homocysteine analogs function as competitive inhibitors (London and Gabel, 2001). Finally, a new class of uncompetitive inhibitors of GGT has also been reported (King et al., 2009; Wickham et al., 2012, 2013). Structures of bacterial GGT and human GGT with inhibitors bound provide insight into the mechanism of inhibition of the enzyme (Ida et al., 2014; Terzyan et al., 2015; Wada et al., 2008, 2010).

10.17.2.2.7 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 (Pandur et al., 2007; Chikhi et al., 1999; Reuter et al., 2009; DeNicola et al., 2011). GGT is expressed in many tumors and contributes to resistance to chemotherapy (Hanigan et al., 1999a,b; Pompella 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; Lee et al., 2007). Studies in animal models indicate that inhibitors of GGT may be useful in the treatment of asthma and reperfusion injury (Yamamoto et al., 2011; Tuzova et al., 2014). 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).

10.17.2.2.8 γ -Glutamyl leukotrienase

As noted earlier, Heisterkamp et al. (1991) identified a GGT-related gene, *GGT-rel*, in a human cDNA library. The product of this gene (i.e., GGT-rel) was shown to cleave the γ -glutamyl bond of LTC₄ (a glutathione S-conjugate) but not 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. Analysis of the kinetic activity of human GGT-rel demonstrated that it hydrolyzes the γ -glutamyl bond of glutathione and glutathione conjugates (Wickham et al., 2011). The K_m of GGT-rel for the substrates, including GSH, GSSG, and LTC₄, is similar to those exhibited by GGT, but the rate of the GGT-rel reaction is 37-fold slower than that exhibited by GGT, resulting in a second order rate kinetic for human GGT-rel that is 37-fold lower than for GGT (Wickham et al., 2011). A study of LTC₄ metabolism in three patients deficient in GGT showed increased levels of LTC₄ but no detectable level of its metabolite LTD₄ (Mayatepek et al., 2004). These data indicate that in humans, LTC₄ is metabolized primarily by GGT not GGT-rel. Localization of GGT-rel expression in normal human tissues showed it to be expressed by macrophages present in many tissues and cells within the adrenal gland, salivary gland, pituitary, thymus, spleen, liver, bone marrow, small intestine, stomach, testis, prostate, and placenta (Hanigan et al., 2015). However, within tissues that express both GGT and GGT-rel, the enzymes are expressed by different cell types and have access to different substrates. GGT-rel is expressed on cell surfaces that are in contact with blood and intercellular fluids, while GGT1 is expressed on cell surfaces that have access primarily to fluids in ducts and glands throughout the body.

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 leukotrienase, which has since been shown to be the mouse homologue of GGT-rel (Carter et al., 1997). Comparison of kidney homogenates from wild-type and GGT-null mice demonstrated that both GGT and GGT-rel cleave LTC₄ to LTD₄ (Carter et al., 1994; Han et al., 2002). No detailed kinetic studies comparing the activity and substrate specific of mouse GGT and GGT-rel have been published. It has been shown that in the mouse, acivicin inhibits both GGT-rel and GGT, but glycylglycine does not accelerate the velocity of the GGT-rel-catalyzed reaction as is observed for the GGT-catalyzed reaction (Carter et al., 1994, 1997). In mice, GGT-rel is expressed primarily in the spleen (Carter et al., 1994). It is observed on endothelial cells and is also localized to the capillaries and sinusoid throughout the body (Carter et al., 1994). There is also a GGT-rel homologue in the rat (Potdar et al., 1997), although there are no published studies regarding its localization or kinetics.

10.17.2.3 Hydrolysis of Cysteinylglycine and Its Conjugates

At least four peptidases/dipeptidases have been identified to cleave cysteinylglycine and its conjugates, including membrane-bound (aminopeptidase N and cysteinylglycine dipeptidase) and cytosolic (a nonspecific enzyme and leucyl aminopeptidase/prolyl aminopeptidase) enzymes (Poon and Josephy, 2012 and references cited therein). Aminopeptidase N (APN), cysteinylglycine dipeptidase, and leucyl dipeptidase are discussed in the succeeding text.

10.17.2.3.1 Aminopeptidase N (APN)

The sequence of human APN (synonyms, aminopeptidase M, peptidase E, peptidase I, alanine dipeptidase, and alanyl aminopeptidase) was deduced from the corresponding cDNA (Olsen et al., 1988). The cDNA encodes 967 amino acids per subunit, and the sequence contains 11 possible glycosylation sites. Over the last 25 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). They all possess the highly conserved HEXXH(X)18E zinc-binding and GAMEN motifs essential for enzyme activities (Luan et al., 2012). 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 the renal proximal tubules, small intestine, and epididymis (Kozak and Tate, 1982; Tate, 1985, 1989). APN is a homodimer (M_r of the subunit, 130×10^3) that contains two zinc ions per enzyme dimer. The enzyme has a broad specificity toward dipeptides and oligopeptides, amides, and acrylamides. 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 amino acids (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 (Naim et al., 1999; 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).

10.17.2.3.2 Cysteinylglycine dipeptidase

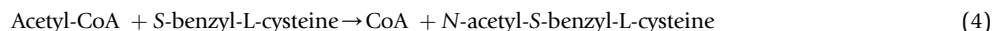
Cysteinylglycine dipeptidase (synonyms, dipeptidase 1, cysteinylglycinase, microsomal dipeptidase, renal dipeptidase, and dehydropeptidase 1) is present in the microvilli of epithelial cells. The enzyme is a dimer (M_r of the monomer $\sim 50 \times 10^3$) and contains four zinc ions per enzyme dimer (Kozak and Tate, 1982; Tate, 1985, 1989; Campbell et al., 1984, 1988). 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 cysteinyl-bis(glycine) and LTD₄ in these membranes (Campbell et al., 1988; Kozak and Tate, 1982; Tate, 1989). The enzyme is also thought to be responsible for the hydrolysis of some β -lactam antibiotics such as perem and carbapem (Campbell et al., 1984). The primary structure of the human cysteinylglycine dipeptidase has been deduced from the cDNA by Adachi et al. (1990a). 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 deduced 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. (1990b) 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, L-cysteinyl-bis(glycine), and S-methyl-L-cysteinylglycine than is APN (Kozak and Tate, 1982).

10.17.2.3.3 Leucyl aminopeptidase

It was originally 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 and 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, and peptidase S; LAP3, LAPEP, and PEPS) (Jösch et al., 2003). These authors provided evidence that leucyl aminopeptidase is the major cysteinylglycine-hydrolyzing activity in the rat liver; leukotriene D₄ is, however, a poor substrate (Jösch et al., 2003). This enzyme also appears to be important in GSH turnover in bovine lens (Cappiello et al., 2004).

10.17.2.4 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. Acetyltransferase activity toward alkyl cysteine S-conjugates was detected in rat liver slices by Barnsley et al. (1969). Subsequently, an acetyltransferase that N-acetylates cysteine S-conjugates (Eq. 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 the pig kidney (Aigner et al., 1996; 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_{max} values were more varied.

The gene coding for the predominant cysteine S-conjugate N-acetyltransferase in the kidney and liver is NAT8 (N-acyltransferase 8) (Veiga-da-Cunha et al., 2010). Interestingly, Veiga-da-Cunha et al. (2010) found that NAT8 homologues are present in all vertebrate genomes sequenced to the time of publication, where they are often encoded by multiple, tandemly repeated genes that encode xenobiotic metabolizing enzymes.

10.17.3 Interorgan Transport of Mercapturates and Mercapturate Intermediates

Formation of glutathione S-conjugates in the body generally occurs mostly in the liver (Dekant et al., 1988, 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, other mammals, such as guinea pigs and humans, have much higher levels of hepatic GGT (Hinchman and Ballatori, 1990; Hinchman et al., 1991, 1993, 1998), and therefore,

metabolic pathways and trafficking of mercapturate intermediates may vary from species to species. We begin with an overview of interorgan 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 the rat liver is via canicular transporters into the bile (e.g., Akerboom et al., 1991; Hinchman et al., 1998; Keppler and König, 2000; Vore, 1993). The importance of the biliary excretion system is underscored by experiments in which 1-naphthylisothiocyanate (ANIT) was 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). Biliary cannulation protects rats from the nephrotoxic effects of hexachloro-1,3-butadiene and *p*-aminophenol (Garland et al., 1990; Nash et al., 1984). This finding also emphasizes the importance of the biliary excretion as a first step in complex multi-organ pathways involved in glutathione S-conjugate and cysteine S-conjugate metabolism (Dekant et al., 1994).

A number of compounds in addition to ANIT, including isothiocyanates and isocyanates (e.g., methyl isocyanate), can react reversibly with GSH and thereby be disposed throughout the body (Baillie and Kassahun, 1994). This ability of the glutathione S-conjugate to act as a dispersant of methyl isocyanate, which is widely used in the chemical industry, is worrisome because of the risk to workers of accidental exposure (which was tragically observed in an industrial accident in Bhopal, India, that resulted in a exposure of a large number of people to methyl isocyanate and many deaths (Samarth et al., 2013; Mishra et al., 2015)). Methyl isocyanate has been shown to target mitochondria and induce chromosomal damage in human kidney epithelial cells in culture (Mishra et al., 2009).

After secretion into the bile, glutathione S-conjugates may arrive intact at the small intestine, or they may be sequentially degraded to the corresponding γ -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 relatively 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 ion, and a thiol-containing fragment by bacterial cysteine S-conjugate β -lyases. The thiol metabolite may be converted to the thiomethyl derivative by thiol S-methyltransferase. 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 "Sulfur Oxidation of Components of the Mercapturate Pathway" and "Thiomethyl Shunt sections." Some cysteine S-conjugates formed in the bile may reenter liver cells to be converted therein to the corresponding mercapturate.

Glutathione S-conjugate and cysteine S-conjugate 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(s) in the microvilli of the brush border of the enterocytes (Tate and Meister, 1985) ensures that at least some of the glutathione S-conjugates are 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 (Grafström et al., 1979). However, in other cases, some glutathione S-conjugates 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 to the GSH transporter (Dekant et al., 1994).

GSTs are widespread among different tissues. 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. Studies with isolated perfused rat kidneys revealed efficient renal removal of the glutathione S-conjugate and cysteine S-conjugate of hexachlorobutadiene (Schrenk et al., 1988a,b). Removal of the conjugates by the kidneys was associated with toxicity. The nephrotoxicity was blocked by the GGT inhibitor AT-125 ((1- α S,5S)- α -amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) and by aminooxyacetate (AOA), indicating the involvement of GGT and a cysteine S-conjugate β -lyase, respectively, in the nephrotoxicity of hexachlorobutadiene S-conjugates (Schrenk et al., 1988a; see "Role of the Mercapturate Pathway and Cysteine S-Conjugate β -Lyases in the Bioactivation of Toxic Halogenated Alkenes" section).

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 tubule cells can also be excreted into the lumen of the tubule (Kramer et al., 1987). The glutathione S-conjugate is metabolized at the brush-border membranes by GGT and APN/dipeptidase (Hughey et al., 1978; Jones et al., 1979). In this process, the glutamate is released to generate a cysteine S-conjugate and glycine (Curthoys and Hughey, 1979). The cysteine S-conjugates produced in the tubules (and S-conjugates arising from tissues other than the kidney reaching the tubular lumen via glomerular filtration) may be transported into the renal epithelial cells from the glomerular filtrate by active and passive uptake mechanisms (Anders, 2004). Both Na^+ -dependent and Na^+ -independent transporters have been implicated in the renal uptake of S-(1,2-dichlorovinyl)-L-cysteine (DCVC, the cysteine S-conjugate of trichloroethylene) (e.g., Anders, 2004; Lash and Anders, 1989; Schaeffer and Stevens, 1987a,b). Most of these studies have focused on uptake of DCVC across the basolateral (peritubular) membrane of the renal proximal cells. Wright et al. (1998) showed that DCVC is taken up by a Na^+ -dependent transport system in isolated renal brush-border membrane vesicles. Several neutral amino

acids but especially phenylalanine, cysteine, and leucine compete with uptake of DCVC across the luminal membrane in this system (Wright et al., 1998).

The probenecid-sensitive organic ion transporter on the basolateral side of the proximal tubular cells appears to play the most important role in the accumulation of S-conjugates and in the renal toxicity of certain halogenated conjugates (Dekant et al., 1994; Pombrio et al., 2001). The transporter has high affinity for haloalkene-derived mercapturates but is also active with glutathione S-conjugates and cysteine S-conjugates with lipophilic attachments to the sulfur (Lash and Anders, 1989; Ullrich et al., 1989). Probenecid protects against the nephrotoxicity of certain haloalkene-derived S-conjugates in vivo, probably by blocking the uptake of the mercapturate (Zhang and Stevens, 1989). Indeed, probenecid protects isolated kidney cells from toxic mercapturates derived from haloalkenes and hydroquinones (Koob et al., 1990).

Once within the renal cells, the cysteine S-conjugates may have three fates (Dekant et al., 1994). They may be 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 that 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, ammonium ion, and a fragment consisting of the original electrophile modified to contain an –SH group (“The Cysteine S-Conjugate β-Lyase Reaction” section). As indicated in the foregoing discussion, once formed in the kidney or transported to the kidney, mercapturates are excreted in the urine. Mercapturates may also be released from the kidneys to the general circulation. However, mercapturates are also capable of being cleaved to cysteine S-conjugates by acylases within the kidney (see “Aminoacylase-Catalyzed Deacetylation of Mercapturic Acids” section). The major interorgan pathways involved in the disposition of mercapturate and mercapturate intermediates are shown in Fig. 3.

Work over the last two decades 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 multidrug resistance protein 1 (MRP1, ABCC1) and MRP2 (ABCC2) (Cole and Deeley, 2006; Nies and Keppler, 2007; Sibhatu et al., 2008; Slot et al., 2008; Cole, 2014). The ABCC2 transporter is important for the transport of LTC₄ (Jedlitschky and Keppler, 2002; Nies and Keppler, 2007; Rosenberg et al., 2010). Additional ABCC members, including long MRP6, short MRP4, MRP5, and 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

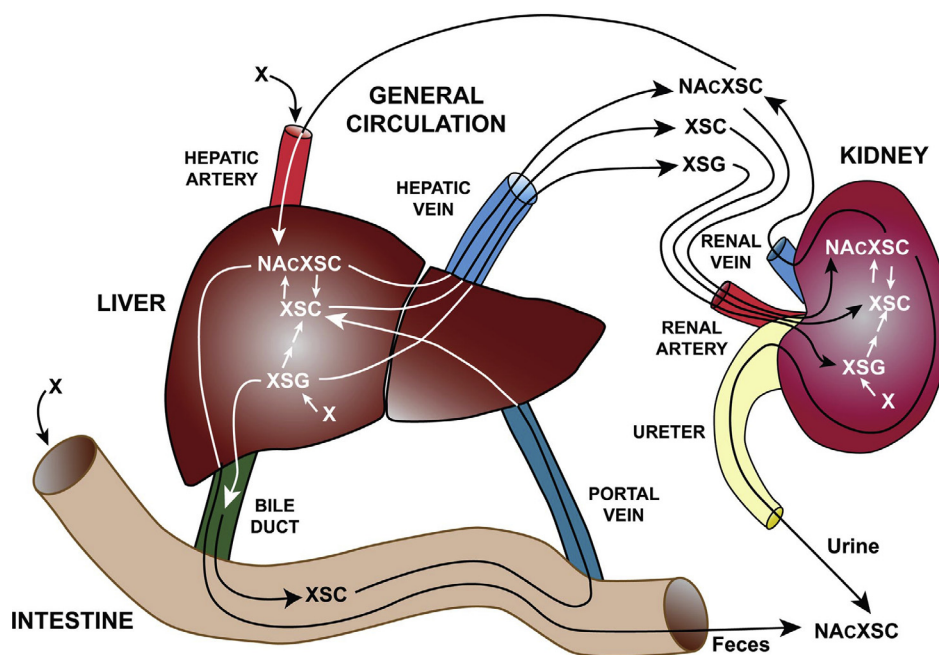


Fig. 3 Interorgan processing of intermediates in the mercapturate pathway in mammals. The relative contribution of various organs to the generation of each component will depend in part on (1) tissue distribution of enzymes involved (which in turn may depend on species and gender), (2) relative transport systems (not shown), and (3) the nature of the electrophile. X, xenobiotic or endogenous electrophile; XSG, glutathione S-conjugate; XSC, cysteine S-conjugate; and NAcXSC, N-acetyl-cysteine S-conjugate (mercapturate). For additional details, see Heuner et al. (1991), Hinchman et al. (1998), Inoue et al. (1981, 1982, 1984a,b), Jösch et al. (1998, 2003), Silbernagl and Heuner (1993), and Rebbear et al. (1998).

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) present evidence that ABCC2 is the major transporter for the elimination of glutathione S-conjugate, glucuronic acid conjugate and sulfate conjugate of various toxins and carcinogens from hepatocytes into the 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 detoxification 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. Solute 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 and in the removal of potentially harmful xenobiotics.

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).

Recently, it was shown that MRP1, 4, and 5 are expressed in the lens, and it was suggested that these proteins are important in the detoxification of potentially toxic metabolites via excretion of the corresponding glutathione S-conjugates (Umaphathy et al., 2015). Evidently, transporters are crucial factors in the detoxification/removal of endogenous and exogenous electrophiles via the mercapturate pathway.

10.17.4 Biotransformations of Endogenous Compounds through the Mercapturate Pathway

10.17.4.1 Deactivation/Detoxification

The most well-characterized example of the involvement of the mercapturate pathway in the metabolism of endogenous compounds relates to the sequential transformations of leukotrienes: LTA₄ → LTC₄ (glutathione S-conjugate) → LTD₄ (cysteinylglycine S-conjugate) → LTE₄ (cysteine S-conjugate) by the consecutive actions of LTC₄ synthase, γ -glutamyl leukotrienase, and cysteinylglycine dipeptidase (Ago et al., 2007; Hagmann et al., 1986; Heisterkamp et al., 2008; Murphy and Gijón, 2007; Örming et al., 1986; Veiga-da-Cunha et al., 2010). LTE₄ (along with LTC₄ and LTD₄) is the slowly reacting substance causing anaphylaxis. LTE₄ is a potent stimulator of renal blood flow and glomerular filtration (Allen and Gellai, 1990; Gulbins et al., 1991). LTC₄ and LTD₄ are rapidly converted to LTE₄ in vivo (Moore et al., 1992). LTE₄ is deactivated in vivo by conversion to the *N*-acetyl derivative (mercapturate) in the kidney tubules and is excreted (Fauler et al., 1991).

LTC₄ synthase is a member of the GST family that is not involved in detoxification per se but rather catalyzes an important biosynthetic reaction (Ago et al., 2007). LTC₄ synthase is present in eosinophils, mast cells, monocytes, neutrophils, and macrophages (Söderström et al., 1988). The mouse and human LTC₄ synthases have been characterized (Söderström et al., 1988). LTC₄ synthase is a membrane-bound enzyme (homodimer, subunit M_r 18 × 10³) that shares some homology with microsomal GST-1 and GST-3 (Lam, 2003). The terminus contains a protein kinase C consensus phosphorylation sequence, raising the possibility that the enzyme is phosphoregulated. It has recently been suggested that drugs targeting LTC₄ synthase may be beneficial for treatment of inflammatory diseases associated with overexpression of this enzyme (Kleinschmidt et al., 2015).

LTC₄ is formed intracellularly, but LTD₄ and LTE₄ are formed extracellularly by the action of the transferase and dipeptidase on the cell surface. Most effects of the leukotrienes are exerted via interactions with specific receptors on the cell surface (Ishikawa, 1993). Two cell membrane receptors have been well characterized, namely, CysLT1 (synonyms, cysteinyl leukotriene receptor 1, cysteinyl leukotriene D4 receptor, and LTD4 receptor) and CysLT2 (Kanaoka and Boyce, 2004; Singh et al., 2010). CysLTR1 belongs to the G-protein coupled receptor 1 family. CysLT1 is the receptor that participates in cysteinyl leukotriene-mediated bronchoconstriction of individuals with and without asthma. Stimulation by LTD4 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 phosphatidylinositol-calcium second messenger system.

The rank order of affinities for the leukotrienes is LTD4 ($K_d \sim 1$ nM) > LTE4 = LTC4 ($K_d \sim 10$ nM) > LTB4. The CysLT1 receptor is widely expressed with highest levels in spleen and peripheral blood leukocytes. Lower expression occurs in several tissues, such as the lung (mostly in smooth muscle bundles and alveolar macrophages), placenta, small intestine, pancreas, colon, and heart. Selective antagonists of the CysLT1 receptor, such as montelukast (Singulair), zafirlukast (Accolate), and pranlukast (Onon), are used in the treatment of asthma, asthma-related diseases, and a few nonasthma-related diseases (Fanning and Boyce, 2013; Riccioni et al., 2007; Sekioka et al., 2015).

LTC₄ is transported from the intracellular space to the extracellular space by an ATP-dependent transporter with high affinity for LTC₄ (110 nM) (Ishikawa, 1992, 1993; Lam, 2003; Schaub et al., 1991). This transporter (the GS-X pump) is identical to the GSSG transporter. (GSSG is a special case of a glutathione S-conjugate.) In addition to the kidney route for

metabolism of leukotrienes mentioned earlier, 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 MR1P1/ABCC1 transporter ($K_m \sim 3 \mu\text{M}$) (Cole and Deeley, 2006; Nies and Keppler, 2007).

Additional endogenous metabolites carried by the MRP1 carrier include the glutathione S-conjugates of prostaglandin (PG)A₂, 15-deoxy- $\Delta^{12,14}$ -PGJ₂, and hydroxynonenal (Cole and Deeley, 2006).

10.17.4.2 Bioactivation

Although the mercapturate pathway evolved for the detoxification of potentially harmful metabolites and xenobiotics, on occasion, the pathway may result in a toxification (or bioactivation) event. 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, 2002; Vauzour et al., 2008, 2010). These *o*-quinones react readily with GSH and cysteine to form catecholamine glutathione S-conjugates 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 the human brain (Spencer et al., 1998). Interestingly, the concentrations of these compounds are generally lower in many regions of human postmortem PD brain compared with 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-conjugate and cysteinyl S-conjugate of dopamine to the corresponding mercapturate (Shen et al., 1996). It has been suggested that the dopamine mercapturate augments dopaminergic neurodegeneration (Montine et al., 2000). Previously, Dryhurst and coworkers established that the mercapturate of dopamine and 5-S-cysteinyl-dopamine are potent neurotoxins. The neurotoxicity of 5-S-cysteinyl catecholamines may be due to their further oxidation to benzothiazine species, such as DHBT-1 (dihydrobenzothiazine-1-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylic acid) (Shen et al., 1996, 1997; Vauzour et al., 2008). 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-cysteinyl-dopamine (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 and 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, disruption of other mitochondrial enzymes/complexes of cerebral energy metabolism (e.g., α -ketoglutarate dehydrogenase complex (KGDHC) and pyruvate dehydrogenase complex (PDHC)) may also contribute to decreased energy production in neurodegenerative disease (reviewed by Gibson et al., 2005; Kiss et al., 2013). 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 activity (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 natural and synthetic estrogens. They are readily oxidized to the corresponding *o*-quinones, which in turn are converted to GSH adducts (Monks and Lau, 1994; Wan and O’Brien, 2014). 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 cysteinyl 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). Some evidence suggests that quinones derived from estradiol and specific glutathione S-conjugates of estradiol are potent inhibitors of human GSTM1-1 and GSTA1-1 (Abel et al., 2004).

10.17.5 Metabolic Pathways Diverging From Mercapturate Biosynthesis

10.17.5.1 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 (RH) (Eq. 5) or (2) hydrolytic cleavage of a thioester (Eq. 6).

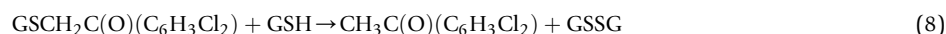
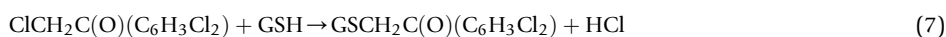


In other cases, the deconjugation may simply be due to reversal of the conjugation reaction; glutathione S-(and possibly cysteine S-) conjugates may then act as vehicles for the delivery of free xenobiotics and GSH throughout the body (Jean et al., 1995). Methyl isocyanate, mentioned earlier, is an example 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. A recently described example in which a glutathione S-conjugate is formed by Michael addition across a reactive double bond followed by reversible cysteine S-conjugate formation involves (6)-shogaol. This compound is a putative chemopreventive agent present in ginger. It has recently been shown that the corresponding cysteine S-conjugate acts as a delivery system for (6)-shogaol to tissues and tumors (Chen et al., 2013).

Baillie and Kassahun (1994) state that "it seems likely that many xenobiotics with carbamate, formamide, urea, or sulfonylurea 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."

10.17.5.2 Deconjugation Coupled to Reductive Dehalogenation

An example of deconjugation coupled to reductive dehalogenation is the nonenzymatic attack of GSH on 2,4-dichlorophenylacetyl chloride (Eq. 7) followed by reaction of the conjugate with a second GSH (Eq. 8) catalyzed by S-phenacylglutathione reductase (Kitada et al., 1985).



The rat liver enzyme catalyzes the reductive dehalogenation of *o*-chloroacetophenone, 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)glutathione 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-dichloroethane 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-(glutathione-S-yl)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. The loss of halide ion yields an episulfonium cysteine S-conjugate (1-(2-amino-2-carboxyethyl)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-(N⁷-guanyl)-glutathione)) with DNA. 1-(2-Amino-2-carboxyethyl)thiiranium is thought to be the cytotoxic species (reviewed by Anders, 2008).

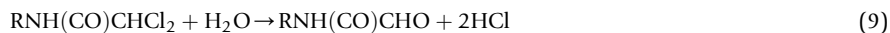
1,2-Dibromo-3-chloropropane was used for many years as a soil fumigant to control nematodes but was banned in the United States in 1977 because it was shown to induce infertility in men and tumors in experimental animals (Clark and Snedeker, 2005). The compound is converted to a glutathione S-conjugate with the loss of bromide at the 1 position, but in this case, deconjugation does not occur. The conjugate is converted to a thiiranium ion with the 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).

10.17.5.3 Hydrolytic Deconjugation

Perhaps the best-studied example occurs in the two-step glyoxalase detoxification reaction. In the first step, GSH forms an adduct with methylglyoxal (synonyms, 2-oxopropanal and pyruvaldehyde), a potentially toxic byproduct of glycolysis (Chakraborty et al., 2014; Allaman et al., 2015), yielding the glutathione S-conjugate S-D-lactoylglutathione in a reaction catalyzed by glyoxalase I. This enzyme is a metalloglutathione S-transferase that catalyzes a 1,2 hydrogen transfer (Creighton and Hamilton, 2001). In the second step, glyoxalase II catalyzes the hydrolytic cleavage of S-D-lactoylglutathione to free GSH and D-lactate (glyoxalase II reaction) (Cordell et al., 2004). Mitochondria require GSH to maintain redox balance, yet GSH is made exclusively in the cytosol. While there are well-defined mitochondrial uptake systems for GSH (Mårtensson et al., 1990; Booty et al., 2015), it has recently been shown that S-D-lactoylglutathione is an alternative source of GSH in mitochondria (Armeni et al., 2014). S-D-Lactoylglutathione is taken up by the mitochondria wherein it is hydrolyzed by glyoxalase II to D-lactate and GSH (Armeni et al., 2014).

In the case of mammalian glyoxalase, both glutathione S-conjugate formation and hydrolytic cleavage are enzyme-catalyzed, and GSH is regenerated. In other cases of hydrolytic deconjugation, the initial formation of the glutathione S-conjugate is enzyme-catalyzed, but the subsequent hydrolysis occurs nonenzymatically. Examples include the net conversion of

chloramphenicol to an aldehyde catalyzed by several GSTs (Eq. 9) (Martin et al., 1980a,b) and dihalomethanes to formaldehyde (Ahmed and Anders, 1978; Anders, 2004, 2008) (Eq. 10).

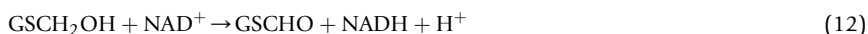


Trypanosomes have a two-enzyme system identical to the mammalian glyoxalase system, except that trypanothione (two glutathione moieties covalently bridged by spermidine) is used in place of GSH. Thus, the glyoxalase system in trypanosomes is a potential drug target (Silva et al., 2008; Wyllie and Fairlamb, 2011; Manta et al., 2013).

Dichloromethane is mutagenic in some systems, and GSH is implicated in the bioactivation (Ahmed and Anders, 1978; Anders, 2008; Schlosser et al., 2015). 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 a hemithioacetal of formaldehyde and can release formaldehyde (Eq. 11). The released formaldehyde may account for the mutagenicity of dichloromethane, but model compounds also suggest a possible role for S-(chloromethyl)glutathione.

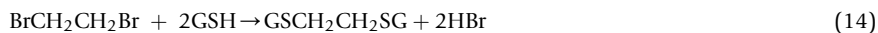


Formaldehyde GSH hemithioacetal is oxidized to S-(formyl)glutathione by a dehydrogenase (Eq. 12). Hydrolytic cleavage of the latter (a thioester) by S-formylglutathione hydrolase yields formic acid and GSH (Eq. 13) (Uotila and Koivusalo, 1997).

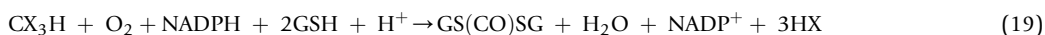
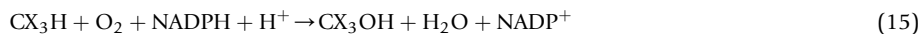


10.17.5.4 Bis(glutathionyl) Conjugate Formation

Trypanothione, mentioned earlier, is a bis-glutathionylated derivative of spermidine. In other cases, some xenobiotics have been shown to 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-bromohydroquinone, 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-(diglutathion-S-yl)-hydroquinone or 2-bromo-3,6-(diglutathion-S-yl)-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 yield the bis(conjugate) (Monks et al., 1985). In a similar reaction, dibromoethane is converted to the diconjugate in the rat liver (Eq. 14) (Nachtomi, 1970).

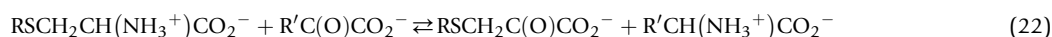
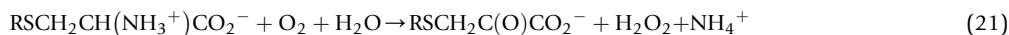


Haloforms are oxidized in a cytochrome P450-catalyzed reaction to trihalomethanol followed by dehydrohalogenation to yield a dihalocarbonyl (Eqs. 15 and 16). Sequential attack by two GSH equivalents yields S-haloformylglutathione (Eq. 17) and the dithiocarbonate (Eq. 18). The net reaction is given by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

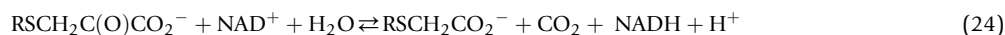
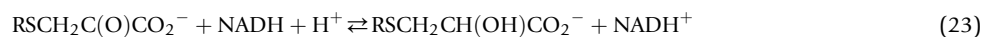


10.17.5.5 Deamination and Transamination

DCVC is oxidized to the corresponding α -keto acid by rat kidney L-amino acid oxidase (Stevens et al., 1986; Eq. 21). Conversion of a cysteine S-conjugate to the corresponding α -keto acid also occurs by transamination with a suitable α -keto acid acceptor (Eq. 22; Commandeur et al., 2000; Cooper et al., 2008a; Cooper et al., 2011; Stevens et al., 1986).



The α -keto acid generated either by the action of L-amino acid oxidase or via transamination may be reduced to the corresponding α -hydroxy acid (Eq. 23). Alternatively, the α -keto acid may be oxidatively decarboxylated in a reaction analogous to the PDHC reaction (Eq. 24).



It is not clear how important these side reactions are to the metabolism of most cysteine S-conjugates in the intact animal, but there is no doubt that they do occur in vivo with some cysteine S-conjugates. For example, methylthioacetate and methylthiolactate are urinary metabolites of S-methyl-L-cysteine in the rat (Barnsley, 1964; Horner and Kuchinskas, 1959). The methyl group of S-methyl-L-cysteine is eventually oxidized to CO_2 (Horner and Kuchinskas, 1959). S-Methyl-L-cysteine is a good aminotransferase substrate of cytosolic rat kidney (Cooper and Meister, 1974, 1981) and human (Cooper et al., 2008a) glutamine transaminase K (GTK). The transamination product is expected to be a precursor of methylthiolactate and methylthioacetate. After administration of S-pentyl-L-cysteine to rabbits, the corresponding α -hydroxy acid and α -keto acid metabolites were found in the urine (James and Needham, 1973). Inorganic sulfate and dicarboxy methyl sulfide are formed in humans and experimental animals administered S-carboxymethylcysteine (Turnbull et al., 1978).

10.17.5.6 Sulfur Oxidation of Components of the Mercapturate Pathway

In some cases, oxidation of the sulfur of the conjugates in the mercapturate pathway to sulfoxides and sulfones occurs in vivo. For example, methylsulfonylacetate, S-methylsulfonylthiolacetate, and methylmercapturate sulfoxide are metabolites of S-methyl-L-cysteine in rats (Barnsley, 1964; Horner and Kuchinskas, 1959; Sklan and Barnsley, 1968). S-Methylcysteine sulfoxide and the sulfoxides of methylthiolacetate and methylmercapturate are found in the urine of humans administered S-methyl-L-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 S-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 β -lyase reaction and thiomethylation (see the next section). In a few cases, a cysteine S-conjugate sulfoxide has been shown to be a substrate of purified cysteine S-conjugate β -lyases yielding pyruvate and ammonium ion (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 (Irving and Elfarra, 2013; Lash et al., 1994, 2003; Ripp et al., 1997; Sausen and Elfarra, 1991; Sausen et al., 1993) and mutagenic (Irving and Elfarra, 2013). The corresponding mercapturate is also nephrotoxic (Irving et al., 2013). The cysteine S-conjugate sulfoxide of tetrachloroethylene ((S-(1,2,2-trichlorovinyl)-L-cysteine, TCVC) was also shown to be nephrotoxic. The toxicity, however, does not appear to be due to a β -elimination of a sulfenic acid (Elfarra and Krause, 2007). 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 monooxygenase 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) (Bimer 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. 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). Some drugs (e.g., certain mustards) are also converted to mercapturate sulfoxides that are found in human bile (Barr et al., 2008; Teichert et al., 2009).

10.17.5.7 Thiomethyl Shunt

As already noted, many cysteine S-conjugates that contain a good leaving group are converted to pyruvate, ammonium ion, 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 $^{14}\text{CO}_2$ and inorganic sulfate from ^{14}C -methyl-labeled S-methyl-L-cysteine (Horner and Kuchinskas, 1959).

Additionally, one other pathway that may lead to inorganic sulfate formation has been described. The cysteine S-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 (Eq. 25), which is oxidized to CO_2 and sulfate (Bakke et al., 1993).



10.17.5.8 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 detoxification end products, the mercapturate itself can sometimes be toxic, for example, in the case of mercapturates derived from halogenated alkenes (Boogard et al., 1989; Commandeur et al., 1991; Newman et al., 2007). 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 ("Background: Bioactivation of Halogenated Alkenes" section).

Several mammalian aminoacylases have been described, including aminoacylase I (AAI, *N*-acyl amino acid hydrolase), aminoacylase II (AAII, aspartoacylase, *N*-acyl-L-aspartate amidohydrolase), acyllysine deacylase (*N*^ε-acyl-L-lysine amidohydrolase), and aminoacylase III (Anders and Dekant, 1994; Newman et al., 2007). AAI 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). AAI activity toward haloalkene-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 AAI 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 AAI shows strong sequence homology to renal dipeptidase and is now assigned to the M20 peptidase family (Liu et al., 2006). Pig kidney AAI, 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. 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 3-D structures of several members of the M20 peptide family, site-directed mutagenesis, and docking studies 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 newer 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.

AAIII is known to exhibit a preference for *N*-acetyl-L-aromatic amino acids. Newman et al. (2007) showed that recombinant mouse AAIII 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_{max}/K_m values for the toxic *N*-acetyl cysteine S-conjugates of halogenated alkenes 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).

AAIII has been less well studied than AAI. The mouse liver enzyme is a mixture of homodimers and homotetramers, both of which are active. A model of the dimer-tetramer interaction has been proposed (Ryazantsev et al., 2007). Recently, it was shown that inhibition of AAIII protects neuronal cells against the toxicity of 4-hydroxynonenal and 4-hydroxynonenal mercapturate (Tsirulnikov et al., 2012).

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 deacylase is higher (Lau et al., 1995). The balance of *N*-deacylase 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).

10.17.6 Cysteine S-Conjugate β -Lyases

10.17.6.1 Background: Bioactivation of Halogenated Alkenes

Haloalkenes (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 United States. Part of the US population is

exposed to haloalkenes from such sites and in the workplace (Wu and Schaum, 2000) and possibly through “recreational” abuse (Marjot and McLeod, 1989). In experimental animals, haloalkenes, some halogenated alkanes, and dichloroacetylene are toxic, especially to the kidneys (e.g., Dekant et al., 1994; Elfara, 1993; Koob and Dekant, 1991). In humans, heavy exposure to trichloroethylene is associated with an increased risk of cancer to the kidney (Brauch et al., 2004; Rusyn et al., 2014; Alanee et al., 2015) and possibly to the liver (Hansen et al., 2013; Vlaanderen et al., 2013), PD (Gash et al., 2008), and damage to the fifth cranial (trigeminal) nerve, sensory nucleus of the brainstem (Buxton and Haywood, 1967), and other nervous tissues (Chiu et al., 2013). Cranial nerve damage appears to be associated with formation of dichloroacetylene from trichloroethylene exposed to an alkaline milieu. Dichloroacetylene is an exceptionally good substrate of microsomal GSTs (Eq. 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*-acetylcysteine 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; Boogard et al., 1989; Dekant, 2001, 2003; Koob and Dekant, 1991; McGoldrick et al., 2003; Lash et al., 2014; Yaqoob et al., 2014; Cristofori et al., 2015). 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 earlier, the mercapturates derived from the halogenated alkenes are toxic because they can be hydrolyzed back to the corresponding cysteine S-conjugate by aminoacylases (e.g., Uttamsingh et al., 2000). Thus, the mercapturate pathway enzymes together with aminoacylases 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; Cooper et al., 2011; 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 and S₂ (*pars convoluta*) region (e.g., Jones et al., 1988) and to isolated human proximal tubules (Chen et al., 1990).

10.17.6.2 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 (“Catalytic Mechanism of the Cysteine S-Conjugate β -Lyase Reaction and Syncatalytic Inactivation” section). 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.

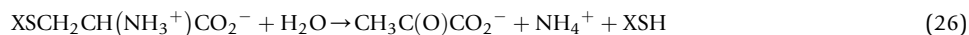
It has been shown that a lethal dose of DCVC in mice leads to inhibition of cell division, inhibition of renal repair, and acute renal failure (Korrapati et al., 2005; Vaidya et al., 2003a,b). However, sublethal (priming) doses are autoprotective by stimulating cell division and renal repair (Korrapati et al., 2006). Acute renal failure in humans is associated with high morbidity and mortality rates. However, renal failure 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 carried out 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. Expression of eighteen common proteins was 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).

10.17.6.3 The Cysteine S-Conjugate β -Lyase Reaction

Historically, 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, Schultze 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 Schultze, 1965; Bhattacharya and Schultze, 1967, 1972). It was known for many years that several electrophilic xenobiotics (e.g., phenacetin, acetaminophen, and *N*-hydroxy-2-acetylaminofluorene) are thiomethylated in vivo. Thiomethylation was originally assumed to involve a sulfonium 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 and thiomethylation.

The thiomethylation pathway (shunt) (see “Thiomethyl Shunt” section) for the detoxification of xenobiotics was first elucidated by Tateishi and colleagues. 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 ion, 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). Tateishi et al. (1978b) coined the name “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 Eq. 26.



The actual products of the enzyme-catalyzed reaction are XSH and aminoacrylate [$\text{CH}_2 = \text{CH}(\text{NH}_3^+)\text{CO}_2^-$]. The latter undergoes nonenzymatic tautomerization to the α -imino [$\text{CH}_3\text{CH}(\text{NH}_2^+)\text{CO}_2^-$] and subsequent hydrolysis to pyruvate ($\text{CH}_3\text{CHOCO}_2^-$) and ammonium ion. When the eliminated sulfur-containing fragment (XSH) is stable (i.e., does not contain moieties that cause XSH to be converted to an extremely reactive electrophile), the –SH group may be methylated as in the case of the sulfur-containing fragment derived from S-(2,4-dinitrophenyl)-cysteine (Tateishi et al., 1978a; Fig. 1, reaction 8; “Thiomethyl Shunt” section). The thiomethylated compound may also be oxidized to the sulfoxide and sulfone as described in “Sulfur Oxidation of Components of the Mercapturate Pathway” section. Alternatively, XSH may be glucuronidated as in the case of 2-benzothiazole derived from benzothiazolyl L-cysteine (BTC) (Elfarra and Hwang, 1990; Fig. 1, reaction 9). Mercapturates and S-glucuronides are readily excreted. Thiomethyl derivatives may be excreted intact or following further oxidation at the sulfur. The fate of chemically reactive XSH fragments is discussed in “Reactive Sulfur-Containing Fragments Generated by β -Lyase Reactions Involving Halogenated Alkene Cysteine S-Conjugates” section.”

10.17.6.4 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). *E. coli* tryptophanase (a “true” physiologically relevant 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 228×10^3 and 70×10^3 and require pyridoxal 5'-phosphate (PLP) as a cofactor. 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 ion. 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 a M_r of about 75×10^3 and is composed of two identical subunits. The enzyme is active with the cysteine S-conjugate of propachlor, *trans*-9-hydroxy-10-(S-(L-cysteinyll))-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 PLP-dependent 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 β -chloroalanine to pyruvate and ammonia, but it is not inactivated in the process. Many PLP-containing enzymes that catalyze this elimination reaction are syncatalytically inactivated by aminoacrylate intermediate(s) formed during the elimination reaction (“Catalytic Mechanism of the Cysteine S-Conjugate β -Lyase Reaction and Syncatalytic Inactivation” section). Finally, the *E. limosum* enzyme exhibits high activity with the S-oxide of propachlor (Larsen and Stevens, 1986). 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 β -lyase activity is widespread in enteric bacteria and that a major portion of cysteine S-conjugates are excreted 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 appears that the cysteine S-conjugate β -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 earlier, 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 and bile duct remains to be determined. Additionally, whether more stable thiol products generated from bacterial 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).

10.17.6.5 Cysteine S-Conjugate β -Lyases in Mammals

In the 1970s, Tateishi et al. (1978b) obtained a highly purified preparation of a cysteine S-conjugate β -lyase from the rat liver, but they did not identify it. The same group also obtained a highly purified preparation of a PLP-dependent cysteine S-conjugate β -lyase from the 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 ion, and *p*-bromophenylmercaptan from S-(*p*-bromophenyl)-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 with S-(*p*-bromophenyl)-L-cysteine is relatively low ($12.3 \text{ nmol min}^{-1} \text{ mg}^{-1}$) compared with the specific activity exhibited by the most active cysteine S-conjugate β -lyases identified thus far (Table 1). Additionally, the 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 β -lyases of rat liver and kidney cytosol, respectively (DCVC as substrate). Over the ensuing decades, many more mammalian cysteine S-conjugate β -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), glutamine transaminase L (GTL)/KAT III, alanine aminotransferase (AlaAT), and cytosolic 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; Cooper et al., 2011). In addition, high- M_r β -lyases occur in both cytosolic and mitochondrial fractions of the rat kidney and, to a lesser extent, rat liver. High- M_r forms in rat kidney cytosol and mitochondria contain GTK and mitAspAT, respectively (Cooper et al., 2011). Of all the mammalian cysteine S-conjugate β -lyases identified thus far, human GTK has the highest inherent specific activity (Table 1; Cooper and Pinto, 2008; Cooper et al., 2011).

10.17.6.6 Cysteine S-Conjugate β -Lyases in Other Organisms

Fungi are known to contain GST activity (Ando et al., 1988; Dowd and Sheehan, 1999) and cysteine S-conjugate β -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 β -lyase pathways needs to be further evaluated.

Table 1 Mammalian PLP-dependent enzymes with L-cysteine S-conjugate β -lyase activity^a

β -Lyase substrates	DCVC	TFEC	BTC	Syncatalytic inactivation	Competing transamination	Approximate specific activity (U/mg ^b)
<i>Enzyme (cytosolic)</i>						
Kynureninase (R)	+	ND	+	+	ND	0.25
GTK/KAT I (R) ^c	+	+	-	-	+	0.6–6.4
GTK/KAT I (H)	+	+	+	-	+	8–40
GTL/KAT III (m)	+	+	-	-	+	0.5–1.1
cytAspAT (R)	+	+	±	+	-	0.04–0.16
AlaAT (P)	+	+	+	+	-	0.004–0.06
BCAT _c (H)	+	+	+	+	-	0.3–0.5
<i>Enzyme (mitochondrial)</i>						
mitAspAT (R)	+	+	+	+	+	0.8–2.3
BCAT _m (H)	+	+	-	+	-	0.2–0.5
AGAT II (R)	+	+	+	+	+	0.2
GABA aminotransferase (P)	ND	+	ND	ND	ND	0.016
High- M_r β -lyase (R) ^d	+	+	+	-	+	1.0–1.2

^aThis table is an update of that of Cooper and Pinto (2006, 2008) and Cooper et al. (2011). See also Pinto et al. (2014) and Cooper et al. (2016). A unit of enzyme activity (U) is defined as the amount of enzyme that catalyzes the formation of 1 μmol of pyruvate per min (usually at 37°C but temperature was not always specified). ND, not determined; R, rat; P, pig; H, human; and m, mouse

^bActivity with DCVC and/or TFEC.

^cHuman liver contains a KAT with strong cysteine S-conjugate β -lyase activity. A cysteine S-conjugate β -lyase has been highly purified from human kidney. The lyase activity copurifies with GTK. The human GTK, unlike the rat enzyme, has activity with BTC. Some GTK activity is also found in rat kidney and liver mitochondria, but the role of mitochondrial GTK as a cysteine S-conjugate β -lyase is uncertain.

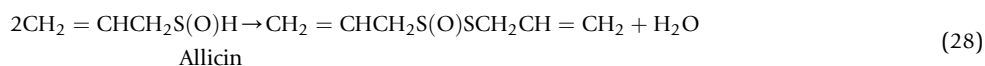
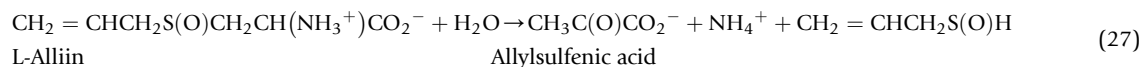
^dActivity is also present in the cytosol.

Three species of parasitic helminths have been shown to contain cysteine S-conjugate β -lyase activity (Adcock et al., 1999). A cysteine S-conjugate β -lyase was purified from the tapeworm *Moniezia expansa* and shown to copurify 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 to yield directly 3-mercaptohexenol. Both pathways are feasible. A series of alkanooates of 3-methyl-3-sulfanylbutan-1-ol ((CH₃)₂C(SH)CH₂CH₂OC(O)R) and a series of alkanooates of 3-sulfanylhexan-1-ol (CH₃CH₂CH₂CH(SH)CH₂CH₂OC(O)R) have been detected in the fruit peel of *Poncirus trifoliata* (a close relative of citrus) (Starkenmann et al., 2007). The authors suggested that the origin of these volatile sulfur-containing compounds is via β -lyase reactions on the corresponding cysteine S-conjugates. Starkenmann and colleagues have documented the importance of cysteine S-conjugate β -lyases in the generation of mercaptans in plants, such as green peppers and allium vegetables, and in mouth bacteria (Starkenmann and Niclass, 2011; Starkenmann et al., 2008, 2011).

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, has been identified as odiferous compounds in human sweat (Natsch et al., 2004). It was suggested that the nonodoriferous 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 L-alliin—a cysteine S-conjugate sulfoxide (Eqs. 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; Cooper et al., 2011). In some cases, the eliminated product is a persulfide, which may be a source of sulfane (S⁰ or S⁰) sulfur, contributing to the anticancer properties of garlic (Pinto et al., 2006).



10.17.6.7 Reactive Sulfur-Containing Fragments Generated by β -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 β -lyase reaction with DCVC (1) gives rise to pyruvate (2), ammonium ion, and a sulfur-containing fragment that has the theoretical structure 1,2-dichloroethylenethiolate (3) (Fig. 4). However, 1,2-dichloroethylenethiolate is extremely unstable and may tautomerize to chlorothioacetyl chloride [ClC(S)CH₂Cl] (not shown) or spontaneously lose 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)

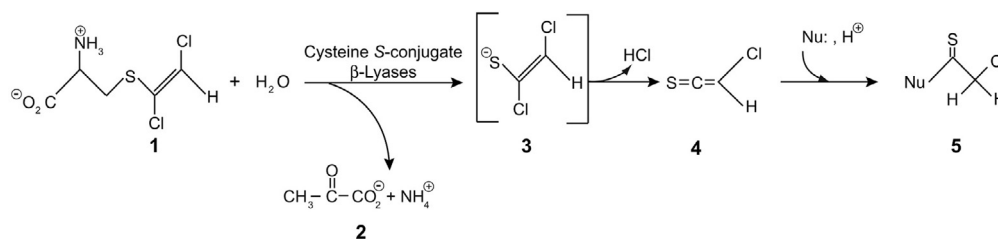


Fig. 4 Bioactivation of *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) by cysteine S-conjugate β -lyases. DCVC (1) is converted to pyruvate (2), ammonium ion, and a sulfur-containing fragment that has the theoretical structure 1,2-dichloroethylenethiolate (3). 1,2-Dichloroethylenethiolate is unstable and in part loses HCl to form the highly reactive chlorothioketene (4). The thioketene reacts with tissue nucleophiles (Nu:) to generate thioacylated products (5). Based in part on Anders (2004, 2008). Reproduced from Cooper, A.J.L., Pinto, J.T., 2008. In Elfarra, A.A. (ed.), *Biotechnology: Pharmaceutical Aspects. Advances in Bioactivation Research*. Springer, New York, NY. pp. 323–346 with permission from Springer.

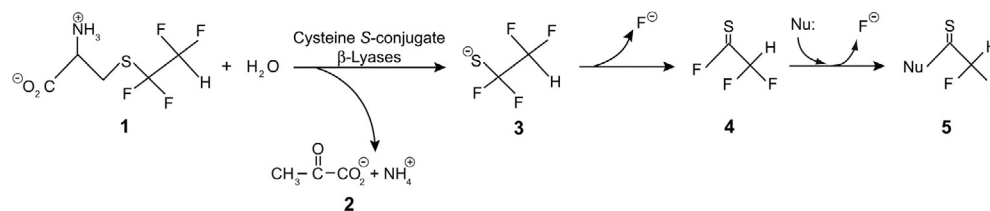


Fig. 5 Bioactivation of *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC) by cysteine *S*-conjugate β -lyases. TFEC (**1**) is converted to pyruvate (**2**), ammonium ion, and a sulfur-containing fragment that has the structure 1,1,2,2-tetrafluoroethanethiolate (**3**). 1,1,2,2-Tetrafluoroethanethiolate (**3**) is unstable and loses F⁻ to form difluorothioacetyl fluoride (**4**). Difluorothioacetyl fluoride reacts with tissue nucleophiles (Nu⁻) to generate thioacylated products (**5**). Based in part on Anders (2004, 2008). Reproduced from Cooper, A.J.L., Pinto, J.T., 2008. In Elfarra, A.A. (ed.), *Biotechnology: Pharmaceutical Aspects. Advances in Bioactivation Research*. Springer, New York, NY. pp 323–346 with permission from Springer.

and proteins (Eyre et al., 1995). This chemistry provides an explanation for the previous finding of Anderson and Schultze (1965), who, as noted earlier, showed covalent incorporation of a sulfur-containing fragment from DCVC to macromolecules via the action of “C–S” lyases.

Cysteine *S*-conjugate β -lyase-catalyzed bioactivation of *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC, the cysteine *S*-conjugate of tetrafluoroethylene) is shown in Fig. 5. The β -lyase reaction with TFEC (**1**) results in the formation of pyruvate (**2**), ammonium ion, and 1,1,2,2-tetrafluoroethanethiolate (**3**). 1,1,2,2-Tetrafluoroethanethiolate is very reactive, losing F⁻ to generate difluorothioacetyl fluoride (**4**), which thioacylates nucleophiles. ³⁵S-labeling studies performed *in vivo* show that phosphatidylethanolamine is a major small-molecular-weight nucleophile thioacylated in mitochondria by the action of cysteine *S*-conjugate β -lyases on TFEC (Hayden et al., 1992). The ϵ -amino group of protein lysyl moieties is 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 *S*-conjugates are nephrotoxic, but unlike the nonbrominated analogs, these compounds are also mutagenic in the Ames test (Finkelstein et al., 1994). Initial studies with the cysteine *S*-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- α -thiolactone) with the loss of F⁻ and Br⁻. The 3-chloro- α -thiolactone was then proposed to undergo conversion to glyoxylate by hydrolysis and the loss of Cl⁻ and H₂S (Finkelstein et al., 1995). Later work from the same group using *o*-phenylenediamine as a trapping agent suggested that a thiirane [2,2-difluoro-3-chlorothiane] was a likely intermediate in the decomposition of 1,1-difluoro-2-bromo-2-chloroethanethiolate (Anders, 2008; Finkelstein et al., 1996).

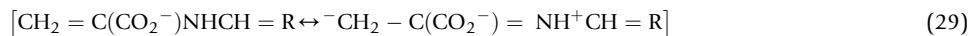
Sevoflurane [(F₃C)₂COCH₂F] is a widely used anesthetic, which may lose HF in the anesthetic circuit to generate 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A), which is nephrotoxic to rats (Anders, 2005). In the rat, Compound A can form two glutathione *S*-conjugates, which are eventually converted to the corresponding cysteine *S*-conjugates. These cysteine *S*-conjugates are substrates of cysteine *S*-conjugate β -lyases giving rise to 2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropanethiol and 2-(fluoromethoxy)-1,1,3,3,3-tetrafluoro-1-propenethiolate, both of which are converted to 2-(fluoromethoxy)-3,3,3-trifluorothionopropanoyl fluoride. Hydrolysis and the loss of HF and H₂S yields 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid. Finally, the 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 indicates that cysteine *S*-conjugate β -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 (2008) for a comprehensive review and original references.

10.17.6.8 Catalytic Mechanism of the Cysteine *S*-Conjugate β -Lyase Reaction and Syncatalytic Inactivation

Table 1 reveals that, with the possible exception of cystathionine γ -lyase, no mammalian cysteine *S*-conjugate β -lyase identified thus far catalyzes a β -lyase reaction as its “natural” physiological reaction. Although cystathionine γ -lyase normally catalyzes a γ -elimination reaction, it can also catalyze β -elimination reactions that may be physiologically relevant. For example, rat liver cystathionine γ -lyase catalyzes (1) the formation of *S*-mercapto-L-cysteine from L-cystine (Cavallini et al., 1960), (2) β -elimination of alkane thiols from several nonhalogenated cysteine *S*-conjugates containing alkyl groups attached to the sulfur (Tomisawa et al., 1988), and (3) β -elimination of alkyl/allyl thiols/persulfides from various alkyl/allyl cysteine *S*-conjugates present in garlic extracts (Cooper and Pinto, 2005; Pinto et al., 2006). Except for kynureninase, the remaining mammalian cysteine *S*-conjugate β -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 (Dolphin et al., 1989; 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 (Eq. 29) (where R = remainder of the quinonoid intermediate) (Stevens, 1985). However, when DCVC binds to the active site, a β -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 carbocation character (Eq. 30) (where R⁺ is the protonated pyridine form of the

intermediate and R is a quinonoid intermediate). In the former case, alanine [$\text{CH}_3\text{CH}(\text{NH}_3^+)\text{CO}_2^-$] is released from the active site. In the latter case, aminoacrylate [$\text{CH}_2 = \text{C}(\text{NH}_3^+)\text{CO}_2^-$] is released, which, as noted earlier, is nonenzymatically converted to pyruvate and ammonium ion.



When transamination competes with the β -lyase reaction, an α -keto acid substrate such as α -keto- γ -methiolbutyrate (KMB) or phenylpyruvate (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 45 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). As noted earlier, 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, $-\text{SCF}_2\text{CF}_2\text{H}$ is a less effective nucleofuge than is $-\text{Cl}$. 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., 2002a) but $\sim 2:1$ for rat kidney GTK (Commandeur et al., 2000). Human BCAT_m 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., 2002a). This difference was ascribed 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 $-\text{SCF}_2\text{CF}_2\text{H}$). The eliminated sulfur-containing fragment is chemically very reactive (previous section). Earlier work had shown that the ϵ -amino groups of lysine residues are susceptible to thioacylation (Fisher et al., 1993; Harris et al., 1992; Hayden and Stevens, 1990; Hayden et al., 1991).

As noted in Table 1, TFEC is both a β -lyase and aminotransferase substrate of mitAspAT; the enzyme is also syncatalytically inactivated by TFEC (Cooper et al., 2011). Inactivation is accompanied by several protein modifications. For example, eliminated fragments (structures 3 and 4, Fig. 5) were found to covalently add to the ϵ -amine of susceptible lysine residues (H_2NR), generating $\text{F}_2\text{CCF}_2\text{NHR}$ and $\text{F}_2\text{CC}(=\text{S})\text{NHR}$, respectively. Interestingly, the initial product of the elimination reaction (aminoacrylate or aminoacrylate in Schiff base linkage to PLP cofactor) was also found to covalently add to a susceptible protein residue. In this case, the addition is to a cysteine residue giving rise to a lanthionine residue (Cooper et al., 2011).

Curiously, both human BCAT_m and BCAT_c 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 BCAT_m and BCAT_c, respectively (Cooper et al., 2003). On the other hand, both rat kidney GTK and mouse GTL 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 amino acids containing a neutral side group, including some 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 or vicinity of the active site.

The earlier discussion attests to the wide scope of *nonphysiological* β -elimination reactions that can give rise to highly reactive aminoacrylate. However, aminoacrylate is also the *physiological* end product of several PLP-catalyzed reactions. Insofar as aminoacrylate takes seconds to many minutes to be nonenzymatically converted to pyruvate and ammonia, the possibility exists that proteins and other macromolecules in the vicinity of the aminoacrylate-generating enzymes will be severely "damaged." It is of considerable interest, therefore, that nature has evolved enzymes (RidA proteins) that are widely distributed and that considerably accelerate the rate of conversion of aminoacrylate to pyruvate and ammonia, thereby lessening the impact of enzyme-generated aminoacrylate (Ernst et al., 2014; Niehaus et al., 2014, 2015).

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

As noted earlier, 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 (e.g., Jones et al., 1988; Cooper et al., 1993; Kim et al., 1997), and (3) the proximal tubules are especially prone to damage from toxic halogenated cysteine S-conjugates; it was quickly assumed that GTK is synonymous with cysteine S-conjugate β -lyase. Indeed, GTK is given this name as a synonym in the human genome directory. The gene for the human enzyme cysteine conjugate beta-lyase 1 (CCBL1) is located on chromosome 9 (Perry et al., 1995). Cysteine conjugate beta-lyase 2 (CCBL2) is also noted to be present in the human genome. We have recently shown that this gene codes for GTL a protein closely related to GTK (Pinto et al., 2014).

Naming GTK and GTL as cysteine S-conjugate β -lyases in the human genome is unfortunate because, as shown in Table 1, other PLP-dependent enzymes catalyze cysteine S-conjugate β -lyase reactions and may also contribute to the formation of reactive fragments from nephrotoxic cysteine S-conjugates. Moreover, the cysteine S-conjugate β -lyase reactions are often not normally physiologically relevant. In the following sections, we focus on GTK and mitAspAT as major cysteine S-conjugate β -lyases in mammals.

10.17.7.1 GTK/Kynurenine Aminotransferase I

The ability of glutamine to participate in enzyme-catalyzed transamination was first discovered by Meister et al. (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 the kidney but well represented in other tissues), and a liver type named glutamine transaminase L (GTL, predominantly in the liver, with lower 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 L-amino acids and α -keto acids. In general, the enzyme has a preference for L-glutamine, L-methionine (and many other sulfur-containing amino acids), L-phenylalanine (and some other aromatic amino acids), and the corresponding α -keto acids. As noted earlier, cysteine S-conjugates are also substrates of rat kidney GTK. Interestingly, L-selenocysteine Se-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 -ketoglutaramate, α KGM) 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 KMB, to salvage the α -keto acid analogs of essential amino acids, and possibly to generate cyclic sulfur-containing imines (reviewed by Cooper, 2004; Pinto et al., 2014; Cooper et al., 2016). In addition, the glutamine transaminases coupled to ω -amidase may provide anaplerotic α -ketoglutarate as an energy source in rapidly dividing cells (Cooper et al., 2016).

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_r of 47×10^3 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 are almost identical. The only difference is 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. (1993). Mosca et al. (1994) assumed that rat kidney KAT (actually KAT I) is identical to cysteine S-conjugate β -lyase and to GTK and that the differences between the sequences obtained by the two groups are 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 the 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).

Human GTK was purified from the kidney tissue (Lash et al., 1990a). Subsequently, human GTK was cloned and sequenced (Goldfarb et al., 1996; Perry et al., 1995). The deduced amino acid sequence of the human enzyme exhibits an 82% overall similarity to the deduced sequence of the rat enzyme with a 90% similarity around the PLP binding site. Recombinant human GTK (rhGTK) is a homodimer (subunit $M_r \sim 47,900$) containing one PLP per monomer (Han et al., 2004; Rossi et al., 2004). Crystallized rhGTK/KAT I 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 earlier, the active site has a high degree of hydrophobicity that allows binding of amino acids and cysteine S-conjugates with large noncharged side groups.

To our knowledge, a possible mitochondrial form of human GTL has not been considered. The GTL 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 acid addition at the N-terminus that contains a mitochondrial-targeting sequence.

10.17.7.2 Mitochondrial Aspartate Aminotransferase

AspAT catalyzes the reversible transamination of glutamate and oxaloacetate to α -ketoglutarate and aspartate. Almost all mammalian tissues/cells investigated, with the notable exception of red blood cells, contain high levels of cytosolic and mitochondrial isoforms of AspAT. This ubiquity attests to the importance of these enzymes in amino acid metabolism. The two enzymes are also 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 α -ketoglutarate link amino acid metabolism to the tricarboxylic acid (TCA) cycle. Coupling of an α -ketoglutarate-/glutamate-linked aminotransferase to AspAT and the glutamate dehydrogenase reaction permits the flow of nitrogen from amino acid, to glutamate, to ammonium ion. When this occurs in the periportal cells of the liver, this flow directs excess amino acid nitrogen toward ammonium ion for urea synthesis. For a review of 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). These enzymes belong to the fold type I family, subclass I. It has long been known that the two isoforms 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; Ubuka et al., 1978). 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., 2002a; Table 1). To the best of our knowledge, the ability of human mitAspAT to catalyze cysteine S-conjugate β -lyase reactions 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.

10.17.8 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). AOA (a general inhibitor of PLP enzymes including aminotransferases) protects against DCVC-induced toxicity (e.g., Beuter et al., 1989; Blackmore et al., 2002; Chen et al., 1990, 1994; Lash et al., 1986a) 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 aminotransferase(s) 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 (Boogard 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., 1990a) 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 earlier, 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).

1,3-Dichloropropene (a soil fumigant) increases the incidence of hepatocellular adenomas in male rats (Klaunig et al., 2015). The cysteine S-conjugate and corresponding mercapturate of both the *cis*- and the *trans*-isomers of 1,3-dichloropropene are cytotoxic to renal proximal tubules and to LLC-PK₁ cells (Anders, 2008; Park et al., 1992). The 1,3-dichloropropene-induced cytotoxicity is inhibited by methimazole but not by AOA, indicating a role for FMO, but not for 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-chloroallyloxythio)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 may 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 (e.g., Birmer et al., 1995; Lash et al., 1998, 2014).

10.17.9 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), (3) lipid peroxidation (Beuter et al., 1989; Chen et al., 1990; Groves et al., 1991), and (4) carbonylation of susceptible proteins (Fan et al., 2014). These effects may be attributable to formation of metabolites derived from a β -lyase reaction since, as mentioned earlier, AOA can 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, the 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 adduction 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, as mentioned earlier, mitAspAT is present in every cell that contains mitochondria. How then can one explain the unusual susceptibility of the kidney to halogenated cysteine S-conjugates? Many factors are presumably involved (Boogard 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/macromolecules, relative K_m values, competition from natural amino acid substrates, absolute level of the enzyme, the presence of the enzyme in mitochondria, and the presence of α -keto substrate to keep the enzyme enzymatically active (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 earlier, toxicity may also occur in the liver and occasionally in neural tissue, presumably as a consequence of the widespread tissue occurrence of cysteine S-conjugate β -lyases.

The question may 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 earlier, GTK activity is present in cytosolic and mitochondrial fractions of the 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 the rat kidney, other cysteine S-conjugate β -lyases presumably play an important role in the mitochondrial damage.

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 kidney mitochondria (Parli et al., 1987) suggests that this enzyme 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 et al. (1993). These authors showed that six kidney mitochondrial proteins are thioacylated after rats are acutely administered a high dose of TFEC. No thioacylation of cytosolic kidney proteins was detected (negating an important role for cytosolic GTK in this process). The six proteins were identified as HSP60, mitHSP70, mitAspAT, aconitase, the E₂k (dihydrolipoamide succinyl transferase) enzyme component of the KGDHC, 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, and PCBC)

inhibited dihydrolipoyl dehydrogenase (E_3). Our findings that mitAspAT catalyzes a cysteine *S*-conjugate β -lyase reaction with TFEC in vitro and is syncatalytically inactivated in the process (Cooper et al., 2002a) are consistent with the in vivo finding that this enzyme in the kidney is thioacylated after administration of TFEC to rats (Cooper et al., 2011). We also showed that a high- M_r cysteine *S*-conjugate β -lyase present in the kidney copurifies with HSP70 (Cooper et al., 2001). As cited earlier, the high- M_r cysteine *S*-conjugate β -lyase in rat kidney mitochondria contains mitAspAT. This finding provides a mechanism for the prior finding of 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 E_2 and E_3 enzyme components of KGDHC (and the E_3 enzyme component of BCKADHC) are thioacylated in kidney mitochondria of rats administered TFEC, the E_2 p (dihydrolipoamide acetyl transferase) and E_3 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 mM TFEC (Park et al., 1999). These findings are remarkable because E_3 is a common component of KGDHC, PDHC, and BCKADHC (and the glycine 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 E_3 enzyme components are bound to the complex via E_2 enzyme components in PDHC, whereas E_3 enzyme components are bound to the complex via E_1 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 within the enzyme complex. In addition, PDHC is not known to be 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 TCA-associated enzymes have been proposed to be arranged in supramolecular complexes (metabolons) that facilitate channeling of substrate from one enzyme to another. For example, mitAspAT has been proposed to be part of a metabolon affiliated or localized with KGDHC (see references cited in Cooper et al., 2002b). Based on our findings (Cooper et al., 2002a, 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., 2002b; Fig. 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 has

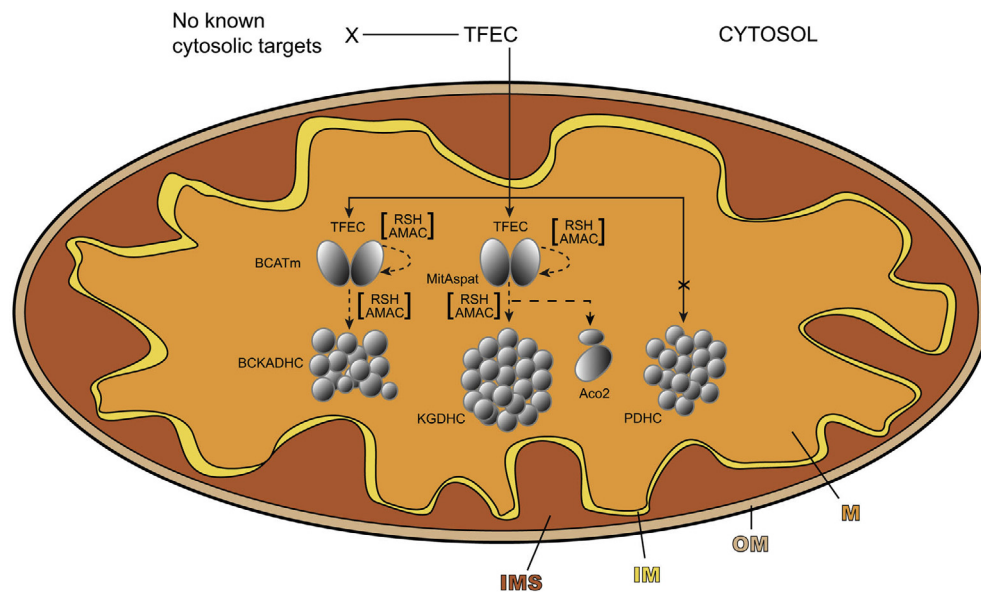


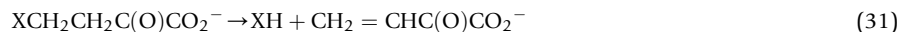
Fig. 6 Model of toxicant channeling of β -lyase-derived TFEC products in mitochondria. TFEC is transported into the mitochondrion where it is converted by cysteine *S*-conjugate β -lyases to aminoacrylate (AMAC) and a reactive sulfur-containing fragment (RSH). AMAC not only is nonenzymatically converted to pyruvate and ammonium ion but also may react with PLP cofactor or susceptible protein residues. The three mitochondrial α -keto acid dehydrogenase complexes (BCKADHC, KGDHC, and PDHC) are represented as multimeric units. Mitochondrial cysteine *S*-conjugate β -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 to KGDHC/acoinitase (Aco 2), respectively, resulting in their inactivation. PDHC is not known to be associated with any aminotransferase/cysteine *S*-conjugate β -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; and M, matrix. Modified from Cooper, A.J.L., Pinto, J.T., 2008. In Elfarra, A.A. (ed.), *Biotechnology: Pharmaceutical Aspects*. Advances in Bioactivation Research. Springer, New York, NY.

been proposed to be part of a metabolon that includes KGDHC and mitAspAT (James et al., 2002; Ovádi and Srere, 2000). Evidence also suggests that substrate channeling occurs between a metabolic unit (termed branched-chain amino acid metabolon) consisting of human BCAT_m and BCKADHC and that the channeling is influenced by the redox state in mitochondria (Islam et al., 2007, 2010). This finding would explain the susceptibility of kidney BCKADHC to TFEC-induced inactivation. Although most of the studies in support of substrate channeling of TCA intermediates have utilized indirect methods, more recent physicochemical methods have provided more direct evidence for TCA substrate channeling (e.g., Wu and Minteer, 2015). The studies of Wu and Minteer (2015) are consistent with the possibility of toxicant channeling.

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 (Korrapati 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 generated by the action of cysteine S-conjugate β-lyase(s) 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.

10.17.10 Homocysteine S-Conjugates

A few toxicological studies have been carried out with the homocysteine S-conjugate analogs of DCVC and BTC (Anders et al., 1988; Lash et al., 1986b, 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 is 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 nonenzymatic β,γ-elimination reaction when converted to the corresponding α-keto acid by an aminotransferase or L-amino acid oxidase (Hollander et al., 1989 and references quoted therein). Activation of the β C–H bond in the α-keto acid (or α-imino acid) facilitates a β,γ-elimination reaction with the production of vinylglyoxylate (2-oxo-3-butenic acid) (Eq. 31). This compound is extremely unstable but can be trapped with a suitable mercaptan (Cooper et al., 1976; Lash et al., 1990b).



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 β-lyase reaction on DCVC is aminoacrylate, which may be less toxic than vinylglyoxylate generated in the β,γ-elimination reaction on the keto analog of DCVHC (Lash et al., 1990b).

Electrophilic estrogen quinones have been shown to react directly with homocysteine to form homocysteine S-conjugates (Gaikwad, 2013). It was suggested that this reaction in vivo could lower the levels of toxic HCys and quench reactive estrogen quinones, resulting in cardiovascular protective effects (Gaikwad, 2013).

10.17.11 Electrophilic Xenobiotics Metabolized through the Mercapturate Pathway

10.17.11.1 General Considerations

Many xenobiotics have been tested for their ability to be detoxified by the mercapturate pathway. A detailed summary was published by Chasseaud (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 aryl nitro compounds, chloro-S-triazines (herbicides), phenoltetrabromophthalens, aralkyl halides, alkyl and alicyclic halides, sulfates and nitro compounds, allyl compounds, alkyl methanesulfonates, organophosphorus compounds, polycyclic aromatic hydrocarbons (via arene oxides), various α,β-unsaturated compounds (esters, aldehydes, ketones, lactones, nitriles, nitro compounds, and 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 documented earlier, 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 is 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/cysteine S-conjugate β-lyase pathway.

10.17.11.2 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 the succeeding text), 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-conjugate 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 (or Pt-S⁻) fragment is proposed to react with macromolecules at thiophilic centers (Zhang and Hanigan, 2003; Fig. 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 cysteine S-conjugate of cisplatin 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 hematologic 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 and *N*-acetyl- β -(*S*-tetrahydrothiophenium)-*L*-alanine in the urine (Hassan and Ehrsson, 1987). Busulfan is converted to a glutathione S-conjugate (*L*- γ -glutamyl- β -(*S*-tetrahydrothiophenium)-*L*-alanyl-glycine) by direct interaction with GSH (Ritter et al., 1999) and enzymatic catalysis by GSTs, especially GSTA1-1 (Czerwinski 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 *L*- γ -glutamyl- β -(*S*-tetrahydrothiophenium)-*L*-alanyl-glycine and to the corresponding mercapturate in the rat (Marchand and Abdel-Monem, 1985; Marchand et al., 1988; Onkenhout et al., 1986).

The busulfan-glutathione adduct undergoes a nonenzymatic β -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 the mercapturate in rats shows that the cysteine S-conjugate must have been generated in vivo either by direct reaction of busulfan with cysteine or via the glutathione S-conjugate. The cysteine S-conjugate of busulfan (β -(*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 β -elimination reaction yielding THT and aminoacrylate should be energetically favorable. Indeed, we have found that (1) homogenates of the rat

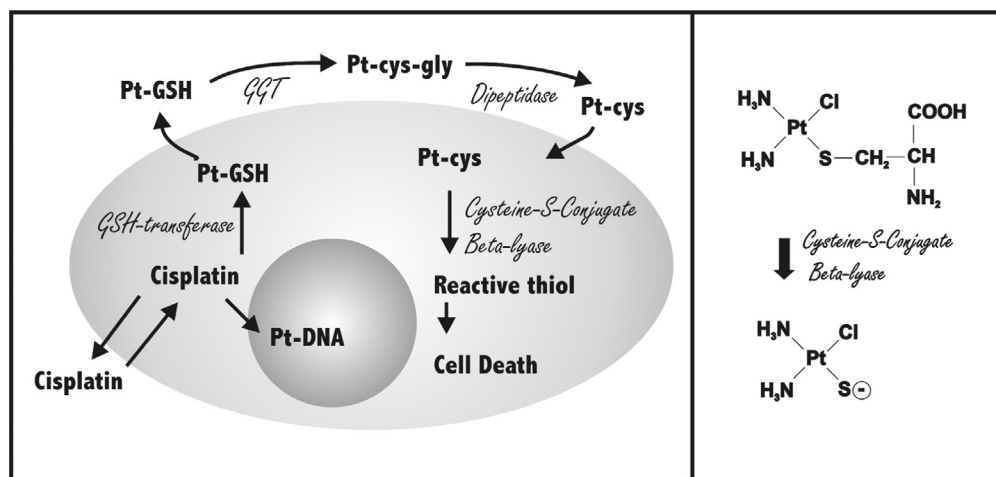


Fig. 7 Bioactivation of cisplatin through the mercapturate/cysteine S-conjugate β -lyase pathway. Cisplatin reacts with GSH in a reaction enhanced by GSTs (glutathione S-transferases). Protection against cisplatin toxicity by acivicin and AOA suggests the involvement of GGT and cysteine S-conjugate β -lyases, respectively, in the bioactivation of cisplatin. The lyase reaction is expected to release a fragment containing a reactive Pt-S bond. Reproduced from Zhang, L., Hanigan, M.H., 2003. Role of cysteine S-conjugate β -lyase in the metabolism of cisplatin. *Journal of Pharmacology and Experimental Therapeutics* 306, 988–994.

liver, kidney, and brain; (2) μM PLP; and (3) highly purified rat liver cystathionine γ -lyase can catalyze a β -elimination reaction with THT-A (Cooper et al., 2008b). A scheme for the possible routes involved in busulfan metabolism is shown in Fig. 8.

It is not clear whether the β -elimination reactions with the busulfan S-conjugates on balance are detoxification or bioactivation events. Formation of THT is probably a detoxification event. However, the formation of aminoacrylate may be a bioactivation event if it leads to selected enzyme inactivation or removal of endogenous thiols. The formation of a GSH analog in which the cysteine of GSH is replaced by a dehydroalanine residue (γ -glutamyldehydroalanyl glycine (EdAG); Fig. 8, structure X) may also be a bioactivation

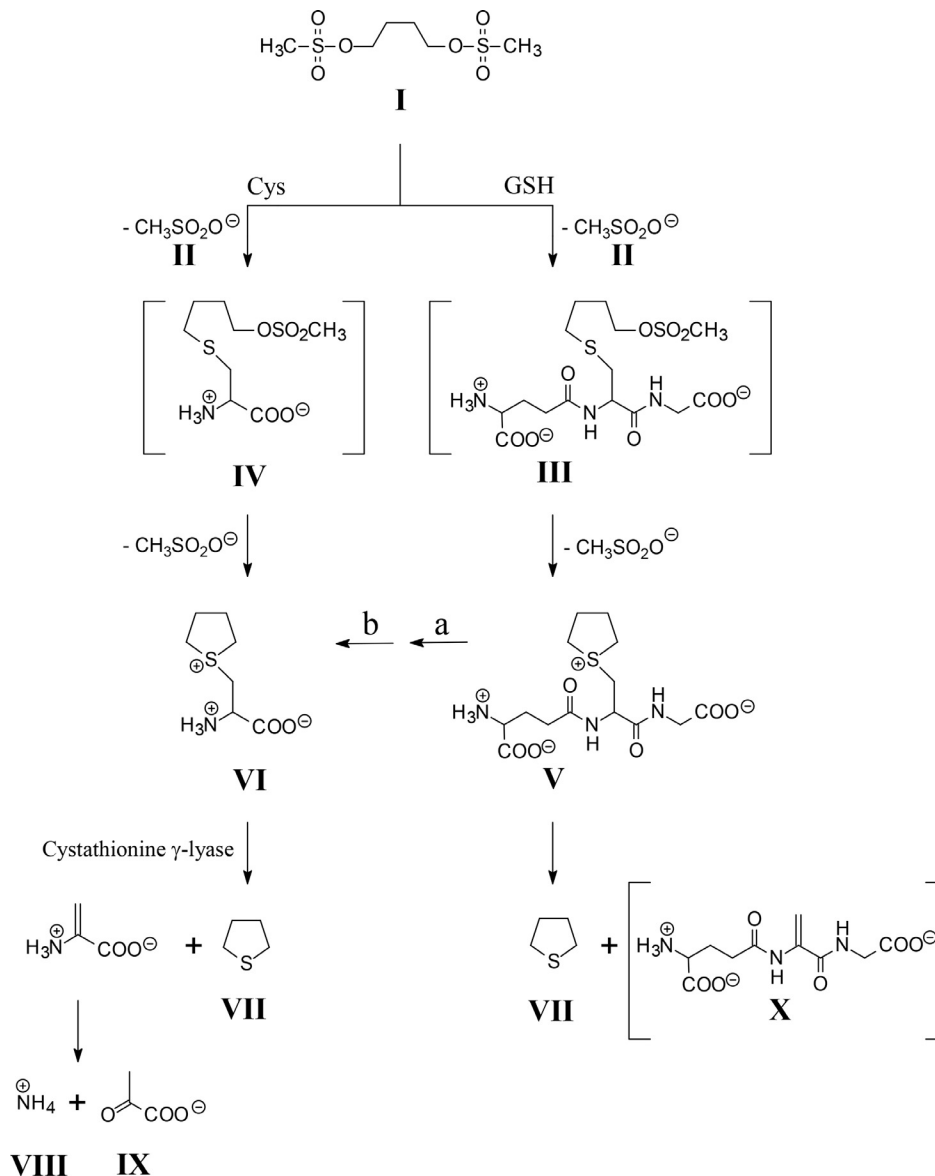


Fig. 8 Proposed mechanism for the metabolic conversion of busulfan to THT. The strong electron-withdrawing properties of the $-\text{OS}(\text{O}_2)\text{CH}_3$ group on busulfan (I) result in electron deficiency at each carbon adjacent to the two methanesulfonyl groups. This deficiency facilitates the nucleophilic attack of the sulfhydryl of GSH on 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 $-\text{OS}(\text{O}_2)\text{CH}_3$ 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. GST (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 ion (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 -glutamyldehydroalanyl glycine (EdAG) (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.

event. It is expected that Michael addition of sulfides across the double bond will occur. In this context, it has been shown that Michael addition of EdAG to GSH results in a GSSG analog in which the disulfide bond of GSSG is replaced by a stable, nonreducing ether bond ((2-amino-5-[[3-[2-[[4-amino-5-hydroxy-5-oxopentanoyl]amino]-3-(carboxymethylamino)-3-oxopropyl]sulfanyl-1-(carboxymethylamino)-1-oxopropan-2-yl]amino]-5-oxopentanoic acid); GSG) (Younis et al., 2008). Thus, formation of EdAG may be deleterious by lowering GSH levels and/or interfering with enzymes that normally utilize GSH/GSSG. It has recently been shown that a reactive cysteine residue in the active site of glutaredoxin 1 (or glutaredoxin 2) (Grx 1/2) reacts with EdAG to form an irreversible thioether (Scian and Atkins, 2015). The enzyme is inactivated in the process. The authors suggested that the adduction of Grxs by EdAG suggests the possible alteration of proteins that are normally regulated via Grx-dependent reversible glutathionylation or deglutathionylation. Dysregulation of Grx-dependent processes could contribute to cellular toxicity of busulfan.

10.17.12 Conclusions

Conversion of an electrophile to the corresponding glutathione S-conjugate and thence to the mercapturate followed by excretion represents a detoxification process. As noted earlier, a very large number of potentially toxic electrophilic xenobiotics are eliminated by this process. In addition, some natural metabolites (e.g., leukotrienes, prostaglandin (PG)A₂, 15-deoxy- $\Delta^{12,14}$ -PG)₂, and hydroxynonenal) may be eliminated as their corresponding mercapturates. However, formation of a glutathione S-conjugate may sometimes lead to bioactivation (toxication). For example, glutathione S-conjugates of some hydroquinones and dihaloethanes may be directly toxic. In other cases, the cysteine 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 sulfoxide may be more toxic than DCVC. 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, the toxicity of the sulfide may be associated with depletion of GSH.

If the cysteine S-conjugate formed in the mercapturate pathway contains a good electron-withdrawing group (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 (although some limited toxicity may be associated with the elimination product aminoacrylate). 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 cisplatin. Some PCBs and aminophenols 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. Throughout life, 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 sulfonium conjugate, can undergo enzyme-catalyzed β -elimination suggests that other drugs or xenobiotics may also undergo similar transformations involving sulfonium conjugates. 1-(Glutathione-S-yl)thiiranium and the corresponding cysteine S-conjugate (1-(2-amino-2-carboxyethyl)thiiranium) formed from dihalopropanes are highly toxic. By analogy with the cysteine S-conjugate β -lyase reaction with the cysteine S-conjugate THT-A, we predict that a β -lyase reaction on 1-(2-amino-2-carboxyethyl)thiiranium will lead to elimination of thiirane, 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 contrast, 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.

Acknowledgments

Work quoted in this article carried out in the authors' laboratories was supported in part by National Institutes of Health grants ES 08421 (AJLC) and CA 57530 (MHH). We thank Dr. Patrick Callery, University of West Virginia, for providing us with Fig. 8 and for his insightful discussions.

See also: 10.16. Glutathione Transferases.

References

- Abel, E. L., Lyon, R. P., Bammler, T. K., Verlinde, C. L., Lau, S. S., Monks, T. J., & Eaton, D. L. (2004). Estradiol metabolites as isoform-specific inhibitors of human glutathione S-transferase. *Chemico-Biological Interactions*, *151*, 21–32.
- Abraham, D. G., Thomas, R. J., & Cooper, A. J. L. (1995). Glutamine transaminase K is not a major cysteine S-conjugate β -lyase of rat kidney: evidence that a high molecular weight lyase fulfills this role. *Molecular Pharmacology*, *48*, 855–860.
- Adachi, H., Tawaragi, Y., Inuzuka, C., Kubota, I., Tsujimoto, M., Nishihara, T., & Nakazato, H. (1990a). Primary structure of human microsomal dipeptidase deduced from molecular cloning. *Journal of Biological Chemistry*, *265*, 3992–3995.
- Adachi, H., Katayama, T., Inuzuka, C., Oikawa, S., Tsujimoto, M., & Nakazato, H. (1990b). Identification of membrane anchoring site of human renal dipeptidase and construction and expression of a cDNA for its secretory form. *Journal of Biological Chemistry*, *265*, 15341–15345.
- Adams, B., Lowpetch, K., Thorndycroft, F., Whyte, S. M., & Young, D. W. (2005). Stereochemistry of reactions of the inhibitor/substrates L- and D-chloroalanine with β -mercaptoethanol catalysed by L-aspartate aminotransferase and D-amino acid aminotransferase respectively. *Organic and Biomolecular Chemistry*, *3*, 3357–3364.
- Adcock, H. J., Brophy, P. M., Teesdale-Spittle, P. H., & Buckberry, L. D. (1999). Cysteine conjugate β -lyase in three species of parasitic helminth. *International Journal for Parasitology*, *29*, 543–548.
- Adcock, H. J., Brophy, P. M., Teesdale-Spittle, P. H., & Buckberry, L. D. (2000). Purification and characterisation of a novel cysteine conjugate β -lyase from the tapeworm *Moniezia expansa*. *International Journal for Parasitology*, *30*, 567–571.
- Ago, H., Kanaoka, Y., Irikura, D., Lam, B. K., Shimamura, T., Austen, K. F., & Miyano, M. (2007). Crystal structure of a human membrane protein involved in cysteinyl leukotriene biosynthesis. *Nature*, *448*, 609–612.
- Ahluwalia, G. S., Grem, J. L., Hao, Z., & Cooney, D. A. (1990). Metabolism and action of amino acid analog anti-cancer agents. *Pharmacology and Therapeutics*, *46*, 243–271.
- Ahmed, A. E., & Anders, M. W. (1978). Metabolism of dichloromethanes to formaldehyde and inorganic halide. II. Studies on the mechanism of the reaction. *Biochemical Pharmacology*, *27*, 2021–2025.
- Aigner, A., Jäger, M., Pasternack, R., Weber, P., Wienke, D., & Wolf, S. (1996). Purification and characterization of cysteine-S-conjugate N-acetyltransferase. *Biochemical Journal*, *317*, 213–218.
- Akerboom, T. P. M., Narayanaswami, V., Kunst, M., & Sies, H. (1991). ATP-dependent S-(2,4-dinitrophenyl)glutathione transport in canalicular plasma membrane vesicles from rat liver. *Journal of Biological Chemistry*, *266*, 13147–13152.
- Alanee, S., Clemons, J., Zahnd, W., Sadowski, D., & Dymda, D. (2015). Trichloroethylene is associated with kidney cancer mortality: a population-based analysis. *Anticancer Research*, *35*, 4009–4013.
- Albiston, A. L., Ye, S., & Chai, S. Y. (2004). Membrane bound members of the MI family: more than aminopeptidases. *Protein and Peptide Letters*, *11*, 491–500.
- Allaman, I., Bélanger, M., & Magistretti, P. J. (2015). Methylglyoxal, the dark side of glycolysis. *Frontiers in Neuroscience*, *9*, 23.
- Allen, D. E., & Gellai, M. (1990). Hemodynamic responses to leukotriene receptor stimulation in conscious rats. *American Journal of Physiology*, *258*, R1034–R1041.
- Anders, M. W. (2004). Glutathione-dependent bioactivation of haloalkanes and haloalkenes. *Drug Metabolism Reviews*, *36*, 583–594.
- Anders, M. W. (2005). Formation and toxicity of anesthetic degradation products. *Annual Review of Pharmacology and Toxicology*, *45*, 147–176.
- Anders, M. W. (2008). Chemical toxicology of reactive intermediates formed by the glutathione-dependent bioactivation of halogen-containing compounds. *Chemical Research in Toxicology*, *21*, 145–159.
- Anders, M. W., & Dekant, W. (1994). Aminoacylases. *Advances in Pharmacology*, *27*, 431–448.
- Anders, M. W., Lash, L., Dekant, W., Efarra, A. A., & Dohn, D. R. (1988). Biosynthesis and biotransformation of glutathione S-conjugates to toxic metabolites. *Critical Reviews in Toxicology*, *18*, 311–341.
- Anderson, P. M., & Schultze, M. O. (1965). Cleavage of S-(1,2-dichlorovinyl)-L-cysteine by an enzyme of bovine origin. *Archives of Biochemistry and Biophysics*, *111*, 593–602.
- Ando, K., Honma, M., Chiba, S., Tahari, S., & Mituzani, J. (1988). Glutathione transferase from *Mucor javanicus*. *Agricultural and Biological Chemistry*, *52*, 135–139.
- Armeni, T., Cianfruglia, L., Piva, F., Urbanelli, L., Luisa Caniglia, M., Pugnaloni, A., & Principato, G. (2014). S-D-Lactoylglutathione can be an alternative supply of mitochondrial glutathione. *Free Radical Biology and Medicine*, *67*, 451–459.
- Awasthi, S., Singhal, S. S., Sharma, R., Zimniak, P., & Awasthi, Y. C. (2003). Transport of glutathione conjugates and chemotherapeutic drugs by RLIP76 (RALBP1): a novel link between G-protein and tyrosine kinase signaling and drug resistance. *International Journal of Cancer*, *106*, 635–646.
- Bailey, J. A., Yavor, A. M., Viggiano, L., Misceo, D., Horvath, J. E., Archidiacono, N., Schwartz, S., Rocchi, M., & Eichler, E. E. (2002). Human-specific duplication and mosaic transcripts: the recent paralogous structure of chromosome 22. *American Journal of Human Genetics*, *70*, 83–100.
- Baillie, T. A., & Kassahun, K. (1994). Reversibility in glutathione-conjugate formation. *Advances in Pharmacology*, *27*, 163–181.
- Bakke, J. E., Larsen, G. L., Aschbacher, P. W., Rafter, J., Gustafsson, J.-Å., & Gustafsson, B. E. (1981). Role of gut microflora in metabolism of glutathione conjugates of xenobiotics. In J. D. Rosen, P. S. Magee, & J. E. Casida (Eds.), *Sulfur in Pesticide Action and Metabolism* (pp. 165–178). Washington, DC: American Chemical Society (ACS Symposium Series No. 158).
- Bakke, J. E., Mulford, D. J., Huwe, J. K., Feil, V. J., & Davison, K. L. (1993). Glutathione-mediated methylthio-turnover and sex differences in the metabolism of pentachloroanisole by rat. *Xenobiotica*, *23*, 949–960.
- Balogh, L. M., Roberts, A. G., Shireman, L. M., Greene, R. J., & Atkins, W. M. (2008). The stereochemical course of 4-hydroxy-2-nonenal metabolism by glutathione S-transferases. *Journal of Biological Chemistry*, *283*, 16702–16710.
- Barnes, M. M., James, S. P., & Wood, P. B. (1959). Formation of mercapturic acid and the levels of glutathione in tissues. *Biochemical Journal*, *71*, 680–690.
- Barnsley, E. A. (1964). The metabolism of S-methyl-L-cysteine in the rat. *Biochimica et Biophysica Acta*, *90*, 24–36.
- Barnsley, E. A., Eskin, N. A. M., James, S. P., & Waring, R. H. (1969). The acetylation of S-alkylcysteines by the rat. *Biochemical Pharmacology*, *18*, 2393–2401.
- Barr, J. R., Pierce, C. L., Smith, J. R., Capacio, B. R., Woolfitt, A. R., Solano, M. I., Wooten, J. V., Lemire, S. W., Thomas, J. D., Ash, D. H., & Ashley, D. L. (2008). Analysis of urinary metabolites of sulfur mustard in two individuals after accidental exposure. *Journal of Analytical Toxicology*, *32*, 10–16.
- Bauman, E., & Preusse, C. (1879). Über bromphenolmercaptursäure. *Berichte der Deutschen Chemischen Gesellschaft*, *12*, 806–810.
- Bernström, K., Larsen, G. L., & Hammerström, S. (1989). Metabolism of leukotriene E₄ to 5-hydroxy-6-mercaptop-7,9-trans-11,14-cis-eicosatetraenoic acid by microfloral cysteine conjugate β -lyase and rat cecum contents. *Archives of Biochemistry and Biophysics*, *275*, 531–539.
- Beuter, W., Cojocel, C., Müller, W., Donaubauer, H. H., & Mayer, D. (1989). Peroxidative damage and nephrotoxicity of dichlorovinylcysteine in mice. *Journal of Applied Toxicology*, *9*, 181–186.
- Bhattacharya, R. K., & Schultze, M. O. (1967). Enzymes from bovine and turkey kidneys which cleave S-(1,2-dichlorovinyl)-L-cysteine. *Comparative Biochemistry and Physiology*, *22*, 723–735.
- Bhattacharya, R. K., & Schultze, M. O. (1972). Properties of DNA treated with S-(1,2-dichlorovinyl)-L-cysteine and a lyase. *Archives of Biochemistry and Biophysics*, *153*, 105–115.
- Binkley, F., & Nakamura, K. (1948). Metabolism of glutathione: hydrolysis by tissues of the rat. *Journal of Biological Chemistry*, *173*, 411–421.
- Birner, G., Werner, M., Ott, M. M., & Dekant, W. (1995). Sex differences in hexachlorobutadiene biotransformation and nephrotoxicity. *Toxicology and Applied Pharmacology*, *132*, 203–212.
- Birner, G., Bernauer, U., Werner, M., & Dekant, W. (1997). Biotransformation and nephrotoxicity of haloalkene-derived cysteines S-conjugates. *Archives of Toxicology*, *72*, 1–8.

- Birner, G., Werner, M., Rosner, E., Mehler, C., & Dekant, W. (1998). Biotransformation, excretion, and nephrotoxicity of the hexachlorobutadiene metabolite (*E*-*N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-*L*-cysteine sulfoxide. *Chemical Research in Toxicology*, *11*, 750–757.
- Blackmore, M., Whitwell, C., Morton, J. K., Gray, T. J., & Lock, E. A. (2002). The effects of haloalkene cysteine conjugates on cytosolic free calcium levels in LLC-PK₁ cells—studies using digital imaging fluorescence microscopy. *Toxicology*, *175*, 143–152.
- Blochberger, T. C., Sabatine, J. M., Lee, Y. C., & Hughey, R. P. (1989). O-Linked glycosylation of rat renal γ -glutamyltranspeptidase adjacent to its membrane anchor domain. *Journal of Biological Chemistry*, *264*, 20718–20722.
- Board, P. G., & Anders, M. W. (2007). Glutathione transferase omega 1 catalyzes the reduction of *S*-(phenacyl)glutathiones to acetophenones. *Chemical Research in Toxicology*, *20*, 149–154.
- Boogaard, P. J., Commandeur, J. N. M., Mulder, G. J., Vermeulen, N. P. E., & Negelkerke, J. F. (1989). Toxicity of the cysteine-*S*-conjugates and mercapturic acids of four structurally related difluoroethylenes in isolated proximal tubular cells from rat kidney. Uptake of the conjugates and activation to toxic metabolites. *Biochemistry and Pharmacology*, *38*, 3731–3741.
- Booty, L. M., King, M. S., Thangaratnarajah, C., Majd, H., James, A. M., Kunji, E. R., & Murphy, M. P. (2015). The mitochondrial dicarboxylate and 2-oxoglutarate carriers do not transport glutathione. *FEBS Letters*, *589*, 621–628.
- Brauch, H., Weirich, G., Klein, B., Rabstein, S., Bolt, H. M., & Bruning, T. (2004). VHL mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference? *Toxicology Letters*, *151*, 301–310.
- Bray, H. G., Franklin, T. J., & James, S. P. (1959). The formation of mercapturic acids. 2. The possible role of glutathione. *Biochemical Journal*, *71*, 690–696.
- Bruschi, S. A., West, K. A., Crabb, J. W., Gupta, R. S., & Stevens, J. L. (1993). Mitochondrial HSP60 (P1 protein) and HSP70-like protein (mortalin) are major targets for modification during *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine-induced toxicity. *Journal of Biological Chemistry*, *268*, 23157–23161.
- Bruschi, S. A., Crabb, J. W., & Stevens, J. L. (1994). The E3 subunit of 2-oxoglutarate, branched-chain α -keto acid, and malate dehydrogenase are adducted during nephrotoxic cysteine-conjugate injury. *Toxicologist*, *14*, 428 (Abstract).
- Bruschi, S. A., Lindsay, J. G., & Crabb, J. W. (1998). Mitochondrial stress protein recognition of inactivated dehydrogenases during mammalian cell death. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 13413–13418.
- Bull, R. J., Ormer, G. A., Cheng, R. S., Stillwell, L., Stauber, A. J., Sasser, L. B., Lingohr, M. K., & Thrall, B. D. (2002). Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. *Toxicology and Applied Pharmacology*, *182*, 55–65.
- Bulle, F., Mattei, M. G., Siegrist, S., Pawlak, A., Passage, E., Chobert, M. N., Laperche, Y., & Guellaën, G. (1987). Assignment of the human γ -glutamyl transferase gene to the long arm of chromosome 22. *Human Genetics*, *76*, 283–286.
- Buxton, P. H., & Haywood, M. (1967). Polyneuritis cranialis associated with industrial trichloroethylene poisoning. *Journal of Neurology, Neurosurgery, and Psychiatry*, *30*, 511–518.
- Campbell, D. J., Forrester, L. J., Zahler, W. L., & Burks, M. (1984). β -Lactamase activity of purified and partially characterized human renal dipeptidase. *Journal of Biological Chemistry*, *259*, 14586–14590.
- Campbell, B. J., Di Shih, Y., Forrester, L. J., & Zahler, W. L. (1988). Specificity and inhibition studies of human renal dipeptidase. *Biochimica et Biophysica Acta*, *956*, 110–118.
- Cappiello, M., Lazzarotti, A., Buono, F., Scalone, A., D'Ambrosio, C., Amodeo, P., Méndez, B. L., Pelosi, P., Del Corso, A., & Mura, U. (2004). New role for leucyl aminopeptidase in glutathione turnover. *Biochemical Journal*, *378*, 35–44.
- Capraro, M. A., & Hughey, R. P. (1985). Use of acivicin in the determination of rate constants for turnover of rat renal γ -glutamyltranspeptidase. *Journal of Biological Chemistry*, *260*, 3408–3412.
- Carter, B. Z., Habib, G. M., Sepulveda, A. R., Barrios, R., Wan, D.-F., Lebovitz, R. M., & Lieberman, M. W. (1994). Type VI RNA is the major γ -glutamyl transpeptidase RNA in the mouse small intestine. *Journal of Biological Chemistry*, *269*, 24581–24585.
- Carter, B. Z., Wiseman, A. L., Orkiszewski, R., Ballard, K. D., Ou, C. N., & Lieberman, M. W. (1997). Metabolism of leukotriene C₄ in γ -glutamyl transpeptidase-deficient mice. *Journal of Biological Chemistry*, *272*, 12305–12310.
- Cavallini, D., DeMarco, C., Mondovi, B., & Mori, B. G. (1960). The cleavage of cystine by cystathionase and the transsulfuration of hypotaurine. *Enzymologia*, *22*, 167–173.
- Cavallini, D., Federici, G., Bossa, F., & Granata, F. (1973). The protective effect of thiosulfate upon the inactivation of aspartate aminotransferase by aminoacrylic-acid-producing substrates. *European Journal of Biochemistry*, *39*, 301–304.
- Cavanagh, J. B., & Buxton, P. H. (1989). Trichloroethylene cranial neuropathy: is it really a toxic neuropathy or does it activate latent herpes virus? *Journal of Neurology, Neurosurgery, and Psychiatry*, *52*, 297–303.
- Chakraborty, S., Karmakar, K., & Chakravorty, D. (2014). Cells producing their own nemesis: understanding methylglyoxal metabolism. *IUBMB Life*, *66*, 667–678.
- Chasseaud, L. F. (1976). Conjugation with glutathione and mercapturic acid excretion. In I. M. Arias, & W. B. Jakoby (Eds.), *Glutathione: Metabolism and Function* (pp. 77–114). New York, NY: Raven Press.
- Chatfield, D. H., & Hunter, W. H. (1973). The metabolism of acetamidothiazoles in the rat. 2-Acetamido-4-chloromethylthiazole. *Biochemical Journal*, *134*, 879–884.
- Chen, J. C., Stevens, J. L., Trifillis, A. L., & Jones, T. W. (1990). Renal cysteine conjugate β -lyase-mediated toxicity studied with primary cultures of human proximal tubular cells. *Toxicology and Applied Pharmacology*, *103*, 463–473.
- Chen, Q., Jones, T. W., & Stevens, J. L. (1994). Early cellular events couple covalent binding of reactive metabolites to cell killing by nephrotoxic cysteine conjugates. *Journal of Cellular Physiology*, *161*, 293–302.
- Chen, Y., Cai, J., Anders, M. W., Stevens, J. L., & Jones, D. P. (2001). Role of mitochondrial dysfunction in *S*-(1,2-dichlorovinyl)-*L*-cysteine-induced apoptosis. *Toxicology and Applied Pharmacology*, *170*, 172–180.
- Chen, H., Soroka, D. N., Zhu, Y., Hu, Y., Chen, X., & Sang, S. (2013). Cysteine-conjugated metabolite of ginger component [6]-shogaol serves as a carrier of [6]-shogaol in cancer cells and in mice. *Chemical Research in Toxicology*, *26*, 976–985.
- Chikhi, N., Holic, N., Guellaën, G., & Laperche, Y. (1999). γ -Glutamyl transpeptidase gene organization and expression: a comparative analysis in rat, mouse, pig and human species. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, *122*, 367–380.
- Chiu, W. A., Jinot, J., Scott, C. S., Makris, S. L., Cooper, G. S., Dzubow, R. C., Bale, A. S., Evans, M. V., Guyton, K. Z., Keshava, N., Lipscomb, J. C., Barone, S., Jr., Fox, J. F., Gwinn, M. R., Schaum, J., & Caldwell, J. C. (2013). Human health effects of trichloroethylene: key findings and scientific issues. *Environmental Health Perspectives*, *121*, 303–311.
- Clark, H. A., & Snedeker, S. M. (2005). Clinical evaluation of the cancer risk of dibromochloropropane (DBCP). *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews*, *23*, 215–260.
- Cole, S. P. (2014). Multidrug resistance protein 1 (MRP1, ABCB1), a "multitasking" ATP-binding cassette (ABC) transporter. *Journal of Biological Chemistry*, *289*, 30880–30888.
- Cole, S. P., & Deeley, R. G. (2006). Transport of glutathione and glutathione conjugates by MRP1. *Trends in Pharmacological Sciences*, *27*, 438–446.
- Coller, B. S., Springer, K. T., Scudder, L. E., Kutok, J. L., Ceruso, M., & Prestwich, G. D. (1993). Substituting isoserine for serine in the thrombin receptor activation peptide SFLLRN confers resistance to aminopeptidase M-induced cleavage and inactivation. *Journal of Biological Chemistry*, *268*, 20741–20743.
- Coloma, J., & Pitot, H. C. (1986). Characterization and sequence of a cDNA clone of γ -glutamyl transpeptidase. *Nucleic Acids Research*, *14*, 1393–1403.
- Colucci, D. F., & Buyske, D. A. (1965). The biotransformation of a sulfonamide to a mercaptan and to mercapturic acid and glucuronide conjugates. *Biochemical Pharmacology*, *14*, 457–466.
- Commandeur, J. N. M., Boogaard, P. J., Mulder, G. J., & Vermeulen, N. P. E. (1991). Mutagenicity and cytotoxicity of two regioisomeric mercapturic acids and cysteine *S*-conjugates of trichloroethylene. *Archives of Toxicology*, *65*, 373–380.
- Commandeur, J. N. M., Andreadou, I., Rooseboom, M., Out, M., De Leur, L. J., Groot, E., & Vermeulen, N. P. E. (2000). Bioactivation of selenocysteine *Se*-conjugates by a highly purified rat renal cysteine conjugate β -lyase/glutamine transaminase K. *Journal of Pharmacology and Experimental Therapeutics*, *294*, 753–761.

- Cooper, A. J. L. (1988). L-Glutamate (2-oxoglutarate) aminotransferases. In E. Kvamme (Ed.), *Glutamine and Glutamate in Mammals* (vol. 1, pp. 123–152). Boca Raton, FL: CRC Press Inc.
- Cooper, A. J. L. (1998). Mechanisms of cysteine S-conjugate β -lyases. *Advances in Enzymology and Related Areas of Molecular Biology*, 72, 199–238.
- Cooper, A. J. L. (2004). The role of glutamine transaminase K (GTK) in sulfur and α -keto acid metabolism in the brain, and the possible bioactivation of neurotoxins. *Neurochemistry International*, 44, 557–577.
- Cooper, A. J. L., & Meister, A. (1974). Isolation and properties of a new glutamine transaminase from rat kidney. *Journal of Biological Chemistry*, 249, 2554–2561.
- Cooper, A. J. L., & Meister, A. (1981). Comparative studies of glutamine transaminases from rat tissues. *Comparative Biochemistry and Physiology*, 69B, 137–145.
- Cooper, A. J. L., & Pinto, J. T. (2005). Aminotransferase, L-amino acid oxidase and β -lyase reactions involving L-cysteine S-conjugates found in allium extracts. Relevance to biological activity? *Biochemical Pharmacology*, 69, 209–220.
- Cooper, A. J. L., & Pinto, J. T. (2006). Cysteine S-conjugate β -lyases. *Amino Acids*, 30, 1–15.
- Cooper, A. J. L., & Pinto, J. T. (2008). Role of cysteine S-conjugate β -lyases in the bioactivation of renal toxicants. In A. A. Elfarra (Ed.), *Biotechnology: Pharmaceutical Aspects. Advances in Bioactivation Research* (pp. 323–346). New York, NY: Springer.
- Cooper, A. J. L., Stephani, R. A., & Meister, A. (1976). Enzymatic reactions of methionine sulfoximine. Conversion to the corresponding α -imino and α -keto acids, and to α -ketobutyrate and methane sulfonamide. *Journal of Biological Chemistry*, 251, 6674–6682.
- Cooper, A. J. L., Abraham, D. G., Gelbard, A. S., Lai, J. C. K., & Petito, C. K. (1993). High activities of glutamine transaminase K (dichlorovinylcysteine β -lyase) and ω -amidase in the choroid plexus of rat brain. *Journal of Neurochemistry*, 61, 1731–1741.
- Cooper, A. J. L., Wang, J., Gartner, C. A., & Bruschi, S. A. (2001). Co-purification of mitochondrial HSP70 and mature protein disulfide isomerase with a functional rat kidney high- M_r cysteine S-conjugate β -lyase. *Biochemical Pharmacology*, 62, 1345–1353.
- Cooper, A. J. L., Bruschi, S. A., Iriarte, A., & Martinez-Carrion, M. (2002a). Mitochondrial aspartate aminotransferase catalyses cysteine S-conjugate β -lyase reactions. *Biochemical Journal*, 368, 253–261.
- Cooper, A. J. L., Bruschi, S. A., & Anders, M. W. (2002b). Toxic, halogenated cysteine S-conjugates and targeting of mitochondrial enzymes of energy metabolism. *Biochemical Pharmacology*, 64, 553–564.
- Cooper, A. J. L., Bruschi, S. A., Conway, M., & Hutson, S. M. (2003). Human mitochondrial and cytosolic branched-chain aminotransferases are cysteine S-conjugate β -lyases, but turnover leads to inactivation. *Biochemical Pharmacology*, 65, 181–192.
- Cooper, A. J. L., Pinto, J. T., Krasnikov, B. F., Niatetskaya, Z. V., Han, Q., Li, J., Vauzour, D., & Spencer, J. P. E. (2008a). Substrate specificity of human glutamine transaminase K as an aminotransferase and as a cysteine S-conjugate β -lyase. *Archives of Biochemistry and Biophysics*, 474, 72–81.
- Cooper, A. J. L., Younis, I. R., Niatetskaya, Z. V., Krasnikov, B. F., Pinto, J. T., Petros, W. P., & Callery, P. S. (2008b). Metabolism of the cysteine S-conjugate of busulfan involves a β -lyase. *Drug Metabolism and Disposition*, 36, 1546–1552.
- Cooper, A. J. L., Krasnikov, B. F., Niatetskaya, Z. V., Pinto, J. T., Callery, P. S., Villar, M. T., Artigues, A., & Bruschi, S. A. (2011). Cysteine S-conjugate β -lyases: important roles in the metabolism of naturally occurring sulfur and selenium-containing compounds, xenobiotics and anticancer agents. *Amino Acids*, 41, 7–27.
- Cooper, A. J. L., Shurubor, Y. I., Dorai, T., Pinto, J. T., Isakova, E. P., Deryabina, Y. I., Denton, T. T., & Krasnikov, B. F. (2016). ω -Amidase: an underappreciated, but important enzyme in L-glutamine and L-asparagine metabolism; relevance to sulfur and nitrogen metabolism, tumor biology and hyperammonemic diseases. *Amino Acids*, 48, 1–20.
- Cordell, P. A., Futers, T. S., Grant, P. J., & Pease, R. J. (2004). The human hydroxyacylglutathione hydrolase (HAGH) gene encodes both cytosolic and mitochondrial forms of glyoxalase II. *Journal of Biological Chemistry*, 279, 28653–28661.
- Correia, M. A., Krowech, G., Caldera-Munoz, P., Yee, S. L., Straub, K., & Castagnoli, N., Jr. (1984). Morphine metabolism revisited. II. Isolation and chemical characterization of a glutathionylmorphine adduct from rat liver microsomal preparations. *Chemico-Biological Interactions*, 51, 13–24.
- Creighton, D. J., & Hamilton, D. S. (2001). Brief history of glyoxalase I and what we have learned about metal ion-dependent, enzyme catalyzed isomerizations. *Archives of Biochemistry and Biophysics*, 387, 1–10.
- Cristofori, P., Sauer, A. V., & Trevisan, A. (2015). Three common pathways of nephrotoxicity induced by halogenated alkenes. *Cell Biology and Toxicology*, 31, 1–13.
- Cummings, B. S., & Lash, L. H. (2000). Metabolism of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells. *Toxicological Sciences*, 53, 458–466.
- Cummings, B. S., Parker, J. C., & Lash, L. H. (2000). Role of cytochrome P450 and glutathione S-transferase α in the metabolism and cytotoxicity of trichloroethylene in rat kidney. *Biochemical Pharmacology*, 59, 531–543.
- Curthoys, N. P., & Hughey, R. P. (1979). Characterization and physiological function of rat renal γ -glutamyl transpeptidase. *Enzyme*, 24, 383–403.
- Czerwinski, M., Gibbs, J. P., & Slattery, J. T. (1996). Busulfan conjugation by glutathione S-transferases γ , μ , and π . *Drug Metabolism and Disposition*, 24, 1015–1019.
- Danielsen, E. M. (1992). Folding of intestinal brush border enzymes. Evidence that high-mannose glycosylation is an essential early event. *Biochemistry*, 31, 2266–2272.
- Daubeuf, S., Accaoui, M. J., Pettersen, I., Huseby, N. E., Visvikis, A., & Galteau, M. M. (2001). Differential regulation of γ -glutamyltransferase mRNAs in four human tumour cell lines. *Biochimica et Biophysica Acta*, 1568, 67–73.
- Davis, M. E. (1988). Effects of AT-125 on the nephrotoxicity of hexachloro-1,3-butadiene in rats. *Toxicology and Applied Pharmacology*, 95, 44–52.
- Davison, C., Rozman, R. S., & Smith, P. K. (1961). Metabolism of bis- β -chloroethyl sulfide (sulfur mustard gas). *Biochemical Pharmacology*, 7, 65–74.
- De Miranda, P., Beacham, L. M., 3rd., Creagh, T. H., & Elion, G. B. (1975). The metabolic disposition of 14 C-azathioprine in the dog. *Journal of Pharmacology and Experimental Therapeutics*, 195, 50–57.
- Dekant, W. (2001). Chemical-induced nephrotoxicity mediated by glutathione S-conjugate formation. *Toxicology Letters*, 124, 21–36.
- Dekant, W. (2003). Biosynthesis of toxic glutathione conjugates from halogenated alkenes. *Toxicology Letters*, 144, 49–54.
- Dekant, W., Lash, L. H., & Anders, M. W. (1988). Fate of glutathione conjugates and bioactivation of cysteine S-conjugates by cysteine conjugate β -lyases. In H. Sies, & B. Ketterer (Eds.), *Glutathione Conjugation: Its Mechanism and Biological Significance* (pp. 415–447). Orlando, FL: Academic Press.
- Dekant, W., Vamvakas, S., & Anders, M. W. (1994). Formation and fate of nephrotoxic and cytotoxic glutathione S-conjugates: cysteine conjugate β -lyase pathway. *Advances in Pharmacology*, 27, 115–162.
- DeNicola, G. M., Karreth, F. A., Humpton, T. J., Gopinathan, A., Wei, C., Frese, K., Mangal, D., Yu, K. H., Yeo, C. J., Calhoun, E. S., Scrimieri, F., Winter, J. M., Hubran, R. H., Iacobuzio-Donahue, C., Kern, S. E., Blair, I. A., & Tuveson, D. A. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature*, 475, 106–109.
- Diederich, M., Wellman, M., Visvikis, A., Puga, A., & Siest, G. (1993). The 5' untranslated region of the human gamma-glutamyl transferase mRNA contains a tissue-specific active translational enhancer. *FEBS Letters*, 332, 88–92.
- Dohn, D. R., Quebbemann, A. J., Borch, R. F., & Anders, M. W. (1985). Enzymatic reaction of chlorotrifluoroethene with glutathione: 19 F NMR evidence for stereochemical control of the reaction. *Biochemistry*, 24, 5137–5143.
- Dolphin, D., Poulson, R., & Avramović, O. (Eds.). (1989). *Glutathione. Chemical, Biochemical and Medicinal Aspects, Part B*. New York, NY: Wiley.
- Dowd, C. A., & Sheehan, D. (1999). Variable expression of glutathione S-transferase isoenzymes in the fungus *Mucor circinelloides*. *FEMS Microbiology Letters*, 170, 13–17.
- Dreessen, B., Westphal, G., Bünger, J., Hallier, E., & Müller, M. (2003). Mutagenicity of the glutathione and cysteine S-conjugates of the haloalkenes 1,1,2-trichloro-3,3,3-trifluoro-1-propene and trichlorotrifluoroethane in the Ames test in comparison with tetrachloroethene analogues. *Mutation Research*, 539, 157–166.
- Duffel, M. W., & Jakoby, W. B. (1982). Cysteine S-conjugate N-acetyltransferase from rat kidney microsomes. *Molecular Pharmacology*, 21, 444–448.
- Duffel, M. W., & Jakoby, W. B. (1985). Cysteine S-conjugate N-acetyltransferase. *Methods in Enzymology*, 113, 516–520.
- Dulik, D. M., Fenselau, C., & Hilton, J. (1986). Characterization of melphalan-glutathione adducts whose formation is catalyzed by glutathione transferases. *Biochemical Pharmacology*, 35, 3405–3409.

- Elfarra, A. A. (1993). Aliphatic halogenated hydrocarbons. In J. B. Hook, & R. S. Goldstein (Eds.), *Toxicology of the Kidney* (2nd ed., pp. 387–414). New York, NY: Raven Press.
- Elfarra, A. A., & Hwang, I. Y. (1990). In vivo metabolites of S-(2-benzothiazolyl)-L-cysteine as markers of in vivo cysteine conjugate β -lyase and thiol glucuronosyl transferase activities. *Drug Metabolism and Disposition*, *18*, 917–922.
- Elfarra, A. A., & Krause, R. J. (2007). S-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide, a reactive metabolite of S-(1,2,2-Trichlorovinyl)-L-cysteine formed in rat liver and kidney microsomes, is a potent nephrotoxicant. *Journal of Pharmacology and Experimental Therapeutics*, *321*, 1095–1101.
- Elfarra, A. A., Jakobsen, I., & Anders, M. W. (1986). Mechanism of S-(1,2-dichlorovinyl)glutathione-induced nephrotoxicity. *Biochemical Pharmacology*, *35*, 283–288.
- Elfarra, A. A., Lash, L. H., & Anders, M. W. (1987). α -Ketoacids stimulate renal cysteine conjugate β -lyase activity and potentiate the cytotoxicity of S-(1,2-dichlorovinyl)-L-cysteine. *Molecular Pharmacology*, *31*, 208–212.
- Engelken, F. J., Bettschart, V., Rahman, M. Q., Parks, R. W., & Garden, O. J. (2003). Prognostic factors in the palliation of pancreatic cancer. *European Journal of Surgical Oncology*, *29*, 368–373.
- Ernst, D. C., Lambrecht, J. A., Schomer, R. A., & Downs, D. M. (2014). Endogenous synthesis of 2-aminoacrylate contributes to cysteine sensitivity in *Salmonella enterica*. *Journal of Bacteriology*, *196*, 3335–3342.
- Eyre, R. J., Stevens, D. K., Parker, J. C., & Bull, R. J. (1995). Renal activation of trichloroethene and S-(1,2-dichlorovinyl)-L-cysteine and cell proliferative response in the kidneys of F344 rats and B6C3F1 mice. *Journal of Toxicology and Environmental Health*, *46*, 465–481.
- Fan, X., Wang, G., English, R. D., & Firoze Khan, M. (2014). Proteomic identification of carbonylated proteins in the kidney of trichloroethene-exposed MRL^{+/+} mice. *Toxicology Mechanisms and Methods*, *24*, 21–30.
- Fanning, L. B., & Boyce, J. A. (2013). Lipid mediators and allergic diseases. *Annals of Allergy, Asthma, and Immunology*, *111*, 155–162.
- Fauler, J., Tsikas, D., Holch, M., Seekamp, A., Nerlich, M. L., Sturm, J., & Frölich, J. C. (1991). Enhanced urinary excretion of leukotriene E₄ by patients with multiple trauma with or without adult respiratory distress syndrome. *Clinical Science*, *80*, 497–504.
- Feil, V. J., Bakke, J. E., Larsen, G. L., & Gustafsson, B. E. (1981). A new metabolite of propachlor isolated from germfree rat excreta. *Biomedical Mass Spectrometry*, *8*, 1–4.
- Finkelstein, M. B., Vamvakas, S., Bittner, D., & Anders, M. W. (1994). Structure-mutagenicity and structure-cytotoxicity studies on bromine-containing cysteine S-conjugates and related compounds. *Chemical Research in Toxicology*, *7*, 157–163.
- Finkelstein, M. B., Dekant, W., Kende, A. S., & Anders, M. W. (1995). α -Thiolactones as novel intermediates in the cysteine conjugate β -lyase-catalyzed bioactivation of bromine-containing cysteine S-conjugates. *Journal of the American Chemical Society*, *117*, 9590–9591.
- Finkelstein, M. B., Dekant, W., & Anders, M. W. (1996). Cysteine conjugate β -lyase-catalyzed bioactivation of bromine-containing cysteine S-conjugates: stoichiometry and formation of 2,2-difluoro-3-haloethiiranes. *Chemical Research in Toxicology*, *9*, 227–231.
- Fisher, M. B., Hayden, P. J., Bruschi, S. A., Dulik, D. M., Yang, Y., Ward, A. J., & Stevens, J. L. (1993). Formation, characterization, and immunoreactivity of lysine thioamide adducts from fluorinated nephrotoxic cysteine conjugates in vitro and in vivo. *Chemical Research in Toxicology*, *6*, 223–230.
- Fitzpatrick, S. M., Cooper, A. J. L., & Duffy, T. E. (1983). Use of β -methylene-DL-aspartate to assess the role of aspartate aminotransferase in cerebral oxidative metabolism. *Journal of Neurochemistry*, *41*, 1370–1383.
- Gaikwad, N. W. (2013). Mass spectrometry evidence for formation of estrogen-homocysteine conjugates: estrogens can regulate homocysteine levels. *Free Radical Biology and Medicine*, *65*, 1447–1454.
- Gall, D. L., Kim, H., Lu, F., Donohue, T. J., Noguera, D. R., & Ralph, J. (2014). Stereochemical features of glutathione-dependent enzymes in the *Sphingobium* sp. strain SYK-6 β -aryl etherase pathway. *Journal of Biological Chemistry*, *289*, 8656–8667.
- Gardell, S. J., & Tate, S. S. (1981). Subunit interactions in γ -glutamyl transpeptidase. *Journal of Biological Chemistry*, *256*, 4799–4804.
- Garland, K. P. R., Eason, C. T., Bonner, F. W., & Nicholson, J. K. (1990). Effects of biliary cannulation and buthionine sulfoximine pretreatment on the nephrotoxicity of *p*-aminophenol in the Fischer 334 rat. *Archives of Toxicology*, *64*, 14–25.
- Gash, D. M., Rutland, K., Hudson, N. L., Sullivan, P. G., Bing, G., Cass, W. A., Pandya, J. D., Liu, M., Choi, D. Y., Hunter, R. L., Gerhardt, G. A., Smith, C. D., Slevi, J. T., & Prince, T. S. (2008). Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity. *Annals of Neurology*, *63*, 184–192.
- Giardina, T., Biagini, A., Dalle Ore, F., Ferre, E., Reynier, M., & Puigserver, A. (1997). The hog intestinal mucosa acylase I: subcellular localization, isolation, kinetic studies and biological function. *Biochimie*, *79*, 265–273.
- Giardina, T., Perrier, J., & Puigserver, A. (2000). The rat kidney acylase I, characterization and molecular cloning. Differences with other acylases. *European Journal of Biochemistry*, *267*, 6249–6255.
- Gibbs, J. P., Czerwinski, M., & Slattery, J. T. (1996). Busulfan-glutathione conjugation catalyzed by human liver cytosolic glutathione S-transferases. *Cancer Research*, *56*, 3678–3681.
- Gibson, G. E., Blass, J. P., Beal, M. F., & Bunik, V. (2005). The α -ketoglutarate-dehydrogenase complex: a mediator between mitochondria and oxidative stress in neurodegeneration. *Molecular Neurobiology*, *31*, 43–63.
- Gietl, Y., Vamvakas, S., & Anders, M. W. (1991). Intestinal absorption of S-(pentachlorobutadienyl)glutathione and S-(pentachlorobutadienyl)-L-cysteine, the glutathione and cysteine S-conjugates of hexachlorobuta-1,3-diene. *Drug Metabolism and Disposition*, *19*, 703–707.
- Goldfarb, P., Perry, S., Harries, H., Scholfield, C., Lock, T., King, L., & Gibson, G. (1996). Molecular cloning and expression of cDNAs for rat and human kidney cysteine conjugate β -lyase. *Biochemical Society Transactions*, *24*, 330S.
- Goodspeed, D. C., Dunn, T. J., Miller, C. D., & Pitot, H. C. (1989). Human γ -glutamyl transpeptidase cDNA: comparison of hepatoma and kidney mRNA in the human and rat. *Gene*, *76*, 1–9.
- Grafström, R., Ormstad, P., Moldéus, P., & Orrenius, S. (1979). Paracetamol metabolism in the isolated perfused rat liver with further metabolism of a biliary paracetamol conjugate by the small intestine. *Biochemical Pharmacology*, *28*, 3573–3579.
- Green, R. M., & Elce, J. S. (1975). Acetylation of S-substituted cysteines by a rat liver and kidney microsomal N-acetyltransferase. *Biochemical Journal*, *147*, 283–289.
- Groves, C. E., Lock, E. A., & Schnellmann, R. G. (1991). Role of lipid peroxidation in renal proximal tubule cell death induced by haloalkene cysteine conjugates. *Toxicology and Applied Pharmacology*, *107*, 54–62.
- Groves, C. E., Hayden, P. J., Lock, E. A., & Schnellmann, R. G. (1993). Differential cellular effects in the toxicity of haloalkene and haloalkene cysteine conjugates to rabbit renal proximal tubules. *Journal of Biochemical Toxicology*, *8*, 49–56.
- Gulbins, E., Parekh, N., Rauterberg, E. W., Schlotmann, K., & Steinhilber, M. (1991). Cysteinyl leukotriene actions on the microcirculation of the normal and split hydronephrotic rat kidney. *European Journal of Clinical Investigation*, *21*, 184–196.
- Hafsa, Z., Tahara, S., & Mizutani, J. (1987). Microbial metabolism of chlorinated nitrobenzenes. IV. Metabolic pathways of 2,4-dichloro-1-nitrobenzene in *Mucor javanicus*. *Journal of Pesticide Science*, *12*, 617–625.
- Hagmann, W., Denzlinger, C., Rapp, S., Weckbecker, G., & Keppler, D. (1986). Identification of the major endogenous leukotriene metabolite in the bile of rats as N-acetyl leukotriene E₄. *Prostaglandins*, *31*, 239–251.
- Han, B., Luo, G., Shi, Z. Z., Barrios, R., Atwood, D., Liu, W., Habib, G. M., Sifers, R. N., Corry, D. B., & Lieberman, M. W. (2002). γ -Glutamyl leukotrienase, a novel endothelial membrane protein, is specifically responsible for leukotriene D₄ formation in vivo. *American Journal of Pathology*, *161*, 481–490.
- Han, Q., Li, J., & Li, J. (2004). pH dependence, substrate specificity and inhibition of human kynurenine aminotransferase I. *European Journal of Biochemistry*, *271*, 4804–4814.
- Han, L., Hiratake, J., Kamiyama, A., & Sakata, K. (2007). Design, synthesis, and evaluation of γ -phosphono diester analogues of glutamate as highly potent inhibitors and active site probes of γ -glutamyl transpeptidase. *Biochemistry*, *46*, 1432–1447.
- Hanigan, M. H. (2014). Gamma-glutamyl transpeptidase: redox regulation and drug resistance. *Advances in Cancer Research*, *122*, 103–141.
- Hanigan, M. H., & Devarajan, P. (2003). Cisplatin: molecular mechanisms. *Cancer Therapy*, *1*, 47–61.
- Hanigan, M. H., & Frierson, H. F., Jr. (1996). Immunohistochemical detection of γ -glutamyl transpeptidase in normal human tissue. *Journal of Histochemistry and Cytochemistry*, *44*, 1101–1108.
- Hanigan, M. H., & Pitot, H. C. (1985). γ -Glutamyl transpeptidase—its role in hepatocarcinogenesis. *Carcinogenesis*, *6*, 165–172.

- Hanigan, M. H., & Ricketts, W. A. (1993). Extracellular glutathione is a source of cysteine for cells that express γ -glutamyl transpeptidase. *Biochemistry*, *32*, 6302–6306.
- Hanigan, M. H., Brown, J. E., & Ricketts, W. A. (1993). γ -Glutamyl transpeptidase, a glutathionase, is present in some cell culture grade bovine sera. *In Vitro Cellular and Developmental Biology. Animal*, *29A*, 831–833.
- Hanigan, M. H., Frierson, H. F., Jr., Swanson, P. E., & De Young, B. R. (1999a). Altered expression of γ -glutamyl transpeptidase in human tumors. *Human Pathology*, *30*, 300–305.
- Hanigan, M. H., Gallagher, B. C., Townsend, D. M., & Gabarra, V. (1999b). γ -Glutamyl transpeptidase accelerates tumor growth and increases the resistance of tumors to cisplatin in vivo. *Carcinogenesis*, *20*, 553–559.
- Hanigan, M. H., Gillies, E. M., Wickham, S., Wakeham, N., & Wirsig-Wiechmann, C. R. (2015). Immunolabeling of gamma-glutamyl transferase 5 in normal human tissues reveals that expression and localization differ from gamma-glutamyl transferase 1. *Histochemistry and Cell Biology*, *143*, 505–515.
- Hansen, J., Sallmén, M., Seldén, A. I., Anttila, A., Pukkala, E., Andersson, K., Bryngelsson, I. L., Raaschou-Nielsen, O., Olsen, J. H., & McLaughlin, J. K. (2013). Risk of cancer among workers exposed to trichloroethylene: analysis of three Nordic cohort studies. *Journal of the National Cancer Institute*, *105*, 869–877.
- Harris, J. W., Dekant, W., & Anders, M. W. (1992). In vivo detection and characterization of protein adducts resulting from bioactivation of halothane cysteine S-conjugates by ^{19}F NMR: chlorotrifluoroethene and tetrafluoroethene. *Chemical Research in Toxicology*, *5*, 34–41.
- Hassal, C. D., Gandolfi, A. J., Duhamel, R. C., & Brendel, K. (1984). The formation and biotransformation of cysteine conjugates of halogenated ethylenes by rabbit renal tubules. *Chemico-Biological Interactions*, *49*, 283–297.
- Hassan, M., & Ehrsson, H. (1987). Urinary metabolites of busulfan in the rat. *Drug Metabolism and Disposition*, *15*, 399–402.
- Hayden, P. J., & Stevens, J. L. (1990). Cysteine conjugate toxicity, metabolism and binding to macromolecules in isolated rat kidney mitochondria. *Molecular Pharmacology*, *37*, 468–476.
- Hayden, P. J., Ichimura, T., McCann, D. J., Pohl, L. R., & Stevens, J. L. (1991). Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial proteins using antibodies raised against halothane metabolite adducts. *Journal of Biological Chemistry*, *266*, 18415–18418.
- Hayden, P. J., Welsh, C. J., Yang, Y., Schaeffer, W. H., Ward, A. J., & Stevens, J. L. (1992). Formation of mitochondrial phospholipid adducts by nephrotoxic cysteine conjugate metabolites. *Chemical Research in Toxicology*, *5*, 232–237.
- Hayes, J. D., Flanagan, J. U., & Jowsey, I. T. (2005). Glutathione transferases. *Annual Review of Pharmacology and Toxicology*, *45*, 51–88.
- Heese, D., Löffler, H. G., & Röhm, K. H. (1988). Further characterization of porcine kidney aminoacylase I reveals close similarity to "renal dipeptidase". *Biological Chemistry Hoppe-Seyler*, *369*, 559–566.
- Heisterkamp, N., Rajpert-De Meyts, E., Uribe, L., Forman, H. J., & Groffen, J. (1991). Identification of a human γ -glutamyl cleaving enzyme related to, but distinct from, γ -glutamyl transpeptidase. *Proceedings of the National Academy of Sciences of the United States of America*, *88*, 6303–6307.
- Heisterkamp, N., Groffen, J., Warburton, D., & Sneddon, T. P. (2008). The human γ -glutamyltransferase gene family. *Human Genetics*, *123*, 321–332.
- Heuner, A., Dekant, W., Schwegler, J. S., & Silbermagl, S. (1991). Localization and capacity of the last step of mercapturic acid biosynthesis and the reabsorption and acetylation of cysteine S-conjugates in the rat kidney. *Pflügers Archiv*, *417*, 523–527.
- Hidalgo, M., Rodríguez, G., Kuhn, J. G., Brown, T., Weiss, G., MacGovren, J. P., Von Hoff, D. D., & Rowinsky, E. K. (1998). A Phase I and pharmacological study of the glutamine antagonist acivicin with the amino acid solution aminosyn in patients with advanced solid malignancies. *Clinical Cancer Research*, *4*, 2763–2770.
- Hinchman, C. A., & Ballatori, N. (1990). Glutathione-degrading capacities of liver and kidney in different species. *Biochemical Pharmacology*, *40*, 1131–1135.
- Hinchman, C. A., Matsumoto, H., Simmons, T. W., & Ballatori, N. (1991). Intrahepatic conversion of a glutathione conjugate to its mercapturic acid: metabolism of 1-chloro-2,4-dinitrobenzene in isolated perfused rat and guinea pig livers. *Journal of Biological Chemistry*, *266*, 22179–22185.
- Hinchman, C. A., Truong, A. T., & Ballatori, N. (1993). Hepatic uptake of intact glutathione S-conjugate, inhibition by organic anions and sinusoidal catabolism. *American Journal of Physiology*, *265*, G547–G554.
- Hinchman, C. A., Rebbore, J. F., & Ballatori, N. (1998). Efficient uptake and concentrative biliary excretion of a mercapturic acid. *American Journal of Physiology*, *275*, G612–G619.
- Hollander, M. M., Reiter, A. J., Horner, W. H., & Cooper, A. J. L. (1989). Conversion of canavanine to α -keto- γ -guanidinoxybutyrate and to vinylglyoxylate and 2-hydroxyguanidine. *Archives of Biochemistry and Biophysics*, *270*, 698–713.
- Horiuchi, S., Inoue, M., & Morino, Y. (1978). γ -Glutamyl transpeptidase sidedness of its active site on renal brush border membrane. *European Journal of Biochemistry*, *87*, 429–437.
- Horner, W. H., & Kuchinskas, E. J. (1959). Metabolism of methyl-labeled S-methylcysteine in the rat. *Journal of Biological Chemistry*, *234*, 2935–2937.
- Hughey, R. P., Rankin, B. B., Elce, J. S., & Curthoys, N. P. (1978). Specificity of a particulate rat renal peptidase and its localization along with other enzymes of mercapturic acid synthesis. *Archives of Biochemistry and Biophysics*, *186*, 211–217.
- Iida, T., Suzuki, H., Fukuyama, K., Hiratake, J., & Wada, K. (2014). Structure of *Bacillus subtilis* gamma-glutamyltranspeptidase in complex with acivicin: diversity of the binding mode of a classical and electrophilic active-site-directed glutamate analogue. *Acta Crystallographica. Section D, Biological Crystallography*, *70*, 607–614.
- Ikeda, Y., Fujii, J., Anderson, M. E., Taniguchi, N., & Meister, A. (1995a). Involvement of Ser-451 and Ser-452 in the catalysis of human γ -glutamyl transpeptidase. *Journal of Biological Chemistry*, *270*, 22223–22228.
- Ikeda, Y., Fujii, J., Taniguchi, N., & Meister, A. (1995b). Expression of an active glycosylated human γ -glutamyl transpeptidase mutant that lacks a membrane anchor domain. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 126–130.
- Inoue, M., Okajima, K., & Morino, Y. (1981). Renal transtubular transport of mercapturic acid in vivo. *Biochimica et Biophysica Acta*, *641*, 122–128.
- Inoue, M., Okajima, K., & Morino, Y. (1982). Metabolic coordination of liver and kidney in mercapturic acid biosynthesis in vivo. *Hepatology*, *2*, 311–316.
- Inoue, M., Okajima, K., & Morino, Y. (1984a). Hepato-renal cooperation in biotransformation, membrane transport, and elimination of cysteine S-conjugates of xenobiotics. *Journal of Biochemistry*, *95*, 247–254.
- Inoue, M., Kinne, R., Tran, T., & Arias, I. M. (1984b). Glutathione transport across hepatocyte plasma membranes. Analysis using isolated brush-border membranes. *European Journal of Biochemistry*, *138*, 491–495.
- Inoue, M., Okajima, K., Nagase, S., & Morino, Y. (1987). Interorgan metabolism and transport of a cysteine-S-conjugate of xenobiotics in normal and albuminemic rats. *Biochemical Pharmacology*, *36*, 2145–2150.
- Irving, R. M., & Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test. *Toxicology*, *306*, 157–161.
- Irving, R. M., Pinkerton, M. E., & Elfarra, A. A. (2013). Characterization of the chemical reactivity and nephrotoxicity of N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine sulfoxide, a potential reactive metabolite of trichloroethylene. *Toxicology and Applied Pharmacology*, *267*, 1–10.
- Ishikawa, T. (1992). The ATP-dependent glutathione S-conjugate export pump. *Trends in Biochemical Sciences*, *17*, 463–468.
- Ishikawa, T. (1993). Glutathione S-conjugate export pump. In K. D. Tew, C. B. Pickett, T. J. Mantle, B. Mannervik, & J. D. Hayes (Eds.), *Structure and Function of Glutathione Transferases* (pp. 211–221). Boca Raton, FL: CRC Press.
- Islam, M. M., Wallin, R., Wynn, R. M., Conway, M., Fujii, H., Mobley, J. A., Chuang, D. T., & Hutson, S. M. (2007). A novel branched-chain amino acid metabolon. Protein-protein interactions in a supramolecular complex. *Journal of Biological Chemistry*, *282*, 11893–11903.
- Islam, M. M., Nautiyal, M., Wynn, R. M., Mobley, J. A., Chuang, D. T., & Hutson, S. M. (2010). Branched-chain amino acid metabolon: interaction of glutamate dehydrogenase with the mitochondrial branched-chain aminotransferase (BCATm). *Journal of Biological Chemistry*, *285*, 265–276.
- Iwamoto, T., Hiraku, Y., Oikawa, S., Mizutani, H., Kojima, M., & Kawanishi, S. (2004). DNA intrastrand cross-link at the 5'-GA-3' sequence formed by busulfan and its role in the cytotoxic effect. *Cancer Science*, *95*, 454–458.
- Iyer, R. A., & Anders, M. W. (1996). Cysteine conjugate β -lyase-dependent biotransformation of the cysteine S-conjugate of the sevoflurane degradation product Compound A in human, nonhuman primate, and rat renal tissue. *Anesthesiology*, *85*, 1454–1461.
- Jakob, M., Miller, Y. E., & Röhm, K. H. (1992). Cloning and sequence analysis of cDNAs encoding aminoacylase I from porcine kidney. *Biological Chemistry Hoppe-Seyler*, *373*, 1227–1231.
- Jakoby, W. B., Stevens, J. L., Duffel, M. W., & Weisiger, R. A. (1984). The terminal enzymes of mercapturate formation and the thiomethyl shunt. *Reviews in biochemical toxicology*, *6*, 97–115.

- James, S. P., & Needham, D. (1973). Some metabolites of *S*-pentyl-L-cysteine in the rabbit and other species. *Xenobiotica*, 3, 207–218.
- James, E. A., Gygi, S. P., Adams, M. L., Pierce, R. H., Fausto, N., Aebersold, R. H., Nelson, S. D., & Bruschi, S. A. (2002). Mitochondrial aconitase modification, functional inhibition, and evidence for a supramolecular complex of the TCA cycle by the renal toxicant *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine. *Biochemistry*, 28, 6789–6797.
- Jean, P. A., Bailie, M. B., & Roth, R. A. (1995). 1-Naphthylisothiocyanate-induced elevation of biliary glutathione. *Biochemical Pharmacology*, 49, 197–202.
- Jedlitschky, G., & Keppler, D. (2002). Transport of leukotriene C₄ and structurally related conjugates. *Vitamins and Hormones*, 64, 163–184.
- John, R. A., & Fasella, P. (1969). The reaction of L-serine *O*-sulfate with aspartate aminotransferase. *Biochemistry*, 8, 4477–4482.
- Jones, D. P., Moldéus, P., Stead, A. H., Ormstad, K., Jörmvall, H., & Orrenius, S. (1979). Metabolism of glutathione and a glutathione conjugate by isolated kidney cells. *Journal of Biological Chemistry*, 254, 2787–2792.
- Jones, T. W., Gerdes, R. G., Ormstad, K., & Orrenius, S. (1985). The formation of both a mono- and a bis-substituted glutathione conjugate of hexachlorobutadiene by isolated rat hepatocytes and following in vivo administration to the rat. *Chemico-Biological Interactions*, 56, 251–267.
- Jones, T. W., Qin, C., Schaeffer, V. H., & Stevens, J. L. (1988). Immunohistochemical localization of glutamine transaminase K, a rat kidney cysteine conjugate β -lyase, and the relationship to the segment specificity of cysteine conjugate nephrotoxicity. *Molecular Pharmacology*, 34, 621–627.
- Jösch, C., Sies, H., & Akerboom, T. P. M. (1998). Hepatic mercapturic acid formation: involvement of cytosolic cysteinylglycine *S*-conjugate dipeptidase activity. *Biochemical Pharmacology*, 56, 763–771.
- Jösch, C., Klotz, L. O., & Sies, H. (2003). Identification of cytosolic leucyl aminopeptidase (EC 3.4.11.1) as the major cysteinylglycine hydrolysing activity in rat liver. *Biological Chemistry*, 384, 213–218.
- Joseph, P. D., Guengerich, F. P., & Miners, J. O. (2005). "Phase I" and "phase 2" drug metabolism: terminology we should phase out? *Drug Metabolism Reviews*, 37, 575–580.
- Joyce-Brady, M., Jean, J. C., & Hughey, R. P. (2001). γ -Glutamyltransferase and its isoform mediate an endoplasmic reticulum stress response. *Journal of Biological Chemistry*, 276, 9468–9477.
- Kanaoka, Y., & Boyce, J. A. (2004). Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. *Journal of Immunology*, 173, 1503–1510.
- Kanhai, W., Dekant, W., & Henschler, D. (1989). Metabolism of the nephrotoxin dichloroacetylene by glutathione conjugation. *Chemical Research in Toxicology*, 2, 51–56.
- Keillor, J. W., Castonguay, R., & Lherbet, C. (2005). γ -Glutamyl transpeptidase substrate specificity and catalytic mechanism. *Methods in Enzymology*, 401, 449–467.
- Keppler, D., & König, J. (2000). Hepatic secretion of conjugated drugs and endogenous substances. *Seminars in Liver Disease*, 20, 265–272.
- Ketterer, B., & Christodoulides, L. G. (1994). Enzymology of cytosolic glutathione *S*-transferases. *Advances in Pharmacology*, 27, 37–69.
- Kim, H. S., Cha, S. H., Abraham, D. G., Cooper, A. J. L., & Endou, H. (1997). Intranephron distribution of cysteine *S*-conjugate β -lyase activity and its implication for hexachloro-1,3-butadiene-induced nephrotoxicity in rats. *Archives of Toxicology*, 71, 131–141.
- King, J. B., West, M. B., Cook, P. F., & Hanigan, M. H. (2009). A novel, species-specific class of uncompetitive inhibitors of γ -glutamyl transpeptidase. *Journal of Biological Chemistry*, 284, 9059–9065.
- Kinlough, C. L., Poland, P. A., Bruns, J. B., & Hughey, R. P. (2005). γ -Glutamyltranspeptidase: disulfide bridges, propeptide cleavage, and activation in the endoplasmic reticulum. *Methods in Enzymology*, 401, 426–449.
- Kishida, K., Saida, N., Yamamura, N., Iwai, Y., & Sasabe, T. (2001). Cysteine conjugate of methazolamide is metabolized by β -lyase. *Journal of Pharmaceutical Sciences*, 90, 224–233.
- Kiss, G., Konrad, C., Coczi, J., Starkov, A. A., Kawamata, H., Manfredi, G., Zhang, S. F., Gibson, G. E., Beal, M. F., Adam-Vizi, V., & Chinopoulos, C. (2013). The negative impact of α -ketoglutarate dehydrogenase complex deficiency on matrix substrate-level phosphorylation. *FASEB Journal*, 27, 2392–2406.
- Kitada, M., McLennan, J. C., & Anders, M. W. (1985). Purification and characterization of *S*-phenacylglutathione reductase from rat liver. *Journal of Biological Chemistry*, 260, 11749–11754.
- Klaunig, J. E., Gehen, S. C., Wang, Z., Klein, P. J., & Billington, R. (2015). Mechanism of 1,3-dichloropropene-induced rat liver carcinogenesis. *Toxicological Sciences*, 143, 6–15.
- Kleinschmidt, T. K., Haraldsson, M., Basavarajappa, D., Lundeberg, E., Thulasingam, M., Ekoff, M., Fauland, A., Lehmann, C., Kahnt, A. S., Lindbom, L., & Haeggström, J. Z. (2015). Tandem benzophenone amino pyridines, potent and selective inhibitors of human leukotriene C₄ synthase. *Journal of Pharmacology and Experimental Therapeutics*, 355, 108–116.
- Koob, M., & Dekant, W. (1990). Metabolism of hexafluoropropene. Evidence for bioactivation by glutathione conjugate formation in the kidney. *Drug Metabolism and Disposition*, 18, 911–916.
- Koob, M., & Dekant, W. (1991). Bioactivation of xenobiotics by formation of toxic glutathione conjugates. *Chemico-Biological Interactions*, 77, 107–136.
- Koob, M., Köchling, A., Weber, S., Lau, S. S., Monks, T. J., & Dekant, W. (1990). Cytotoxicity of 2-bromo-(*N*-acetylcystein-*S*-yl)-hydroquinone isomers in rat renal tubular epithelial cells. *Toxicology Letters*, 53, 143–145.
- Kördel, W., & Schneider, F. (1976). Chemical investigations on pig kidney aminoacylase. *Biochimica et Biophysica Acta*, 445, 446–457.
- Kördel, W., & Schneider, F. (1977). Renal aminoacylase, a zinc enzyme. *Zeitschrift für Naturforschung*, 32C, 342–344.
- Korrapati, M. C., Lock, E. A., & Mehendale, H. M. (2005). Molecular mechanisms of enhanced renal cell division in protection against *S*-1,2-dichlorovinyl-L-cysteine-induced acute renal failure and death. *American Journal of Physiology. Renal Physiology*, 289, F175–F185.
- Korrapati, M. C., Chilakapati, J., Lock, E. A., Latendresse, J. R., Warbritton, A., & Mehendale, H. M. (2006). Preplaced cell division: a critical mechanism of autoprotection against *S*-1,2-dichlorovinyl-L-cysteine-induced acute renal failure and death in mice. *American Journal of Physiology. Renal Physiology*, 291, F439–F455.
- Korrapati, M. C., Chilakapati, J., Witzmann, F. A., Rao, C., Lock, E. A., & Mehendale, H. M. (2007). Proteomics of *S*-(1,2-dichlorovinyl)-L-cysteine-induced renal failure and autoprotection in mice. *American Journal of Physiology. Renal Physiology*, 293, F994–F1006.
- Kozak, E. M., & Tate, S. S. (1982). Glutathione-degrading enzymes of microvillus membranes. *Journal of Biological Chemistry*, 257, 6322–6327.
- Kramer, R. A., Foureman, G., Greene, K. E., & Reed, D. J. (1987). Nephrotoxicity of *S*-(2-chloroethyl)glutathione in the Fischer rat: evidence for γ -glutamyltranspeptidase-independent uptake by the kidney. *Journal of Pharmacology and Experimental Therapeutics*, 242, 741–748.
- Kranendonk, M., Commandeur, J. N. M., Laires, A., Rueff, J., & Vermeulen, N. P. E. (1997). Characterization of enzyme activities and cofactors involved in bioactivation and bioinactivation of chemical carcinogens in the tester strains *Escherichia coli* K12 MX100 and *Salmonella typhimurium* LT2 TA100. *Mutagenesis*, 12, 245–254.
- Kraus, T., Uttamsingh, V., Anders, M. W., & Wolf, S. (2000). Porcine kidney microsomal cysteine *S*-conjugate *N*-acetyltransferase-catalyzed *N*-acetylation of haloalkene-derived cysteine *S*-conjugates. *Drug Metabolism and Disposition*, 28, 440–445.
- Kristiansen, T. Z., Bunkenborg, J., Gronborg, M., Molina, H., Thuluvath, P. J., Argani, P., Goggins, M. G., Maitra, A., & Pandey, A. (2004). A proteomic analysis of human bile. *Molecular & Cellular Proteomics*, 3, 715–728.
- Kubitz, R., & Häussinger, D. (2007). Osmoregulation of bile formation. *Methods in Enzymology*, 428, 313–324.
- Lam, B. K. (2003). Leukotriene C₄ synthase. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 69, 111–116.
- Larsen, G. L., & Bakke, J. E. (1979). Studies on the origin of methylsulfonyl-containing metabolites from propachlor. *Journal of Environmental Science and Health. Part. B*, 14, 495–504.
- Larsen, G. L., & Bakke, J. E. (1981). Enterohepatic circulation in formation of propachlor (2-chloro-*N*-isopropylacetaniilide) metabolites in the rat. *Xenobiotica*, 11, 473–480.
- Larsen, G. L., & Bakke, J. E. (1983). Metabolism of mercapturic acid-pathway metabolites of 2-chloro-*N*-isopropylacetaniilide (propachlor) by gastrointestinal bacteria. *Xenobiotica*, 13, 115–126.
- Larsen, G. L., & Stevens, J. L. (1986). Cysteine conjugate β -lyase in the gastrointestinal bacterium *Eubacterium limosum*. *Molecular Pharmacology*, 29, 97–103.
- Larsen, G. L., Larson, J. D., & Gustafsson, J.-Å. (1983). Cysteine conjugate β -lyase in the gastrointestinal bacterium *Fusobacterium necrophorum*. *Xenobiotica*, 13, 689–700.

- Lash, L. H., & Anders, M. W. (1986). Cytotoxicity of *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine in isolated rat kidney cells. *Journal of Biological Chemistry*, *261*, 13076–13081.
- Lash, L. H., & Anders, M. W. (1989). Uptake of nephrotoxic *S*-conjugates by isolated rat renal proximal tubular cells. *Journal of Pharmacology and Experimental Therapeutics*, *248*, 531–537.
- Lash, L. H., Elfarra, A. A., & Anders, M. W. (1986a). Renal cysteine conjugate β -lyase: bioactivation of nephrotoxic cysteine *S*-conjugates in mitochondrial outer membrane. *Journal of Biological Chemistry*, *261*, 5930–5935.
- Lash, L. H., Elfarra, A. A., & Anders, M. W. (1986b). *S*-(1,2-Dichlorovinyl)-L-homocysteine-induced cytotoxicity in isolated rat kidney cells. *Archives of Biochemistry and Biophysics*, *251*, 432–439.
- Lash, L. H., Nelson, R. M., Van Dyke, R. A., & Anders, M. W. (1990a). Purification and characterization of human kidney cytosolic cysteine conjugate β -lyase activity. *Drug Metabolism and Disposition*, *18*, 50–54.
- Lash, L. H., Elfarra, A. A., Rakiewicz-Nemeth, D., & Anders, M. W. (1990b). Bioactivation of cytotoxic homocysteine *S*-conjugates. *Archives of Biochemistry and Biophysics*, *276*, 322–330.
- Lash, L. H., Sausen, P. J., Duescher, R. J., Cooley, A. J., & Elfarra, A. A. (1994). Roles of cysteine conjugate β -lyase and *S*-oxidase in nephrotoxicity: studies with *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide. *Journal of Pharmacology and Experimental Therapeutics*, *269*, 374–383.
- Lash, L. H., Qian, W., Putt, D. A., Jacobs, K., Elfarra, A. A., Krause, R. J., & Parker, J. C. (1998). Glutathione conjugation of trichloroethylene in rats and mice: sex-, species-, and tissue-dependent differences. *Drug Metabolism and Disposition*, *26*, 12–19.
- Lash, L. H., Putt, D. A., Hueni, S. E., Krause, R. J., & Elfarra, A. A. (2003). Roles of necrosis, apoptosis, and mitochondrial dysfunction in *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide-induced cytotoxicity in primary cultures of human renal proximal tubular cells. *Journal of Pharmacology and Experimental Therapeutics*, *305*, 1163–1172.
- Lash, L. H., Chiu, W. A., Guyton, K. Z., & Rusyn, I. (2014). Trichloroethylene biotransformation and its role in mutagenicity, carcinogenicity and target organ toxicity. *Mutation Research, Reviews in Mutation Research*, *762*, 22–36.
- Lau, S. S., Kleiner, H. E., & Monks, T. J. (1995). Metabolism as a determinant of species susceptibility to 2,3,5-(triglutathion-S-yl)hydroquinone-mediated nephrotoxicity. The role of N-acetylation and N-deacetylation. *Drug Metabolism and Disposition*, *23*, 1136–1142.
- Law, S. H., Redelings, B. D., & Kullman, S. W. (2012). Comparative genomics of duplicate gamma-glutamyl transferase genes in teleosts: medaka (*Oryzias latipes*), stickleback (*Gasterosteus aculeatus*), green spotted pufferfish (*Tetraodon nigroviridis*), fugu (*Takifugu rubripes*), and zebrafish (*Danio rerio*). *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, *318*, 35–49.
- Lee, D. S., Evans, J. C., Robins, S. J., Wilson, P. W., Albano, I., Fox, C. S., Wang, T. J., Benjamin, E. J., D'Agostino, R. B., & Vasan, R. S. (2007). Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *27*, 127–133.
- Li, H., & Dryhurst, G. (1997). Irreversible inhibition of mitochondrial complex I by 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylic acid (DHBT-1): a putative nigral endotoxin of relevance to Parkinson's disease. *Journal of Neurochemistry*, *69*, 1530–1541.
- Li, H., & Dryhurst, G. (2001). Oxidative metabolites of 5-*S*-cysteinyl-dopamine inhibit the pyruvate dehydrogenase complex. *Journal of Neural Transmission*, *108*, 1363–1374.
- Li, H., Shen, X. M., & Dryhurst, G. (1998). Brain mitochondria catalyze the oxidation of 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylic acid (DHBT-1) to intermediates that irreversibly inhibit complex I and scavenge glutathione: potential relevance to the pathogenesis of Parkinson's disease. *Journal of Neurochemistry*, *71*, 2049–2062.
- Lieberman, M. W., Barrios, R., Carter, B. Z., Habib, G. M., Lebovitz, R. M., Rajagopalan, S., Sepulveda, A. R., Shi, Z. Z., & Wan, D. F. (1995). γ -Glutamyl transpeptidase. What does the organization and expression of a multipromoter gene tell us about its functions? *American Journal of Pathology*, *147*, 1175–1185.
- Lieberman, M. W., Wiseman, A. L., Shi, Z. Z., Carter, B. Z., Barrios, R., Ou, C. N., Chévez-Barrios, P., Wang, Y., Habib, G. M., Goodman, J. C., Huang, S. L., Lebovitz, R. M., & Matzuk, M. M. (1996). Growth retardation and cysteine deficiency in γ -glutamyl transpeptidase-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 7923–7926.
- Lindner, H. A., Alary, A., Boju, L. I., Sulea, T., & Ménard, R. (2005). Roles of dimerization domain residues in binding and catalysis by aminoacylase-1. *Biochemistry*, *44*, 15645–15651.
- Liu, Z., Zhen, Z., Zuo, Z., Wu, Y., Liu, A., Yi, Q., & Li, W. (2006). Probing the catalytic center of porcine aminoacylase 1 by site-directed mutagenesis, homology modeling and substrate docking. *Journal of Biochemistry*, *139*, 421–430.
- Livesey, J. C., Anders, M. W., Langvardt, P. W., Putzig, C. L., & Reitz, R. H. (1982). Stereochemistry of the glutathione-dependent biotransformation of vicinal-dihaloalkanes to alkenes. *Drug Metabolism and Disposition*, *10*, 201–204.
- Lock, E. A., & Schnellmann, R. G. (1990). The effect of haloalkene cysteine conjugates on rat renal glutathione reductase and lipoyl dehydrogenase activities. *Toxicology and Applied Pharmacology*, *104*, 180–190.
- Lock, E. A., Sani, Y., Moore, R. B., Finkelstein, M. B., Anders, M. W., & Seawright, A. A. (1996). Bone marrow and renal injury associated with haloalkene cysteine conjugates in calves. *Archives of Toxicology*, *70*, 607–619.
- London, R. E., & Gabel, S. A. (2001). Development and evaluation of a boronate inhibitor of γ -glutamyl transpeptidase. *Archives of Biochemistry and Biophysics*, *385*, 250–258.
- Look, A. T., Ashmun, R. A., Shapiro, L. H., & Peiper, S. C. (1989). Human myeloid plasma membrane glycoprotein CD13(gp150) is identical to aminopeptidase N. *Journal of Clinical Investigation*, *83*, 1299–1307.
- Luan, Y., & Xu, W. (2007). The structure and main functions of aminopeptidase N. *Current Medicinal Chemistry*, *14*, 639–647.
- Luan, Y., Ma, C., Wang, Y., Fang, H., & Xu, W. (2012). The characteristics, functions and inhibitors of three aminopeptidases belonging to the m1 family. *Current Protein and Peptide Science*, *13*, 490–500.
- MacFarlane, M., Schofield, M., Parker, N., Roelandt, C., David, M., Lock, E. A., King, L. J., Goldfarb, P. S., & Gibson, G. G. (1993). Dose-dependent induction of cysteine conjugate β -lyase activity in rat kidney by *N*-acetyl-*S*-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine. *Toxicology*, *77*, 133–144.
- Malherbe, P., Alberati-Giani, D., Köhler, C., & Cesura, A. M. (1995). Identification of a mitochondrial form of kynurenine aminotransferase/glutamine transaminase K from rat brain. *FEBS Letters*, *367*, 141–144.
- Mangold, J. B., & Abdel-Monem, M. M. (1980). Stereoselectivity of the glutathione *S*-transferase catalyzed conjugation of aralkyl halides. *Biochemical and Biophysical Research Communications*, *96*, 47–62.
- Mann, V. M., Cooper, J. M., Krige, D., Daniel, S. E., Schapira, A. H. V., & Marsden, C. D. (1992). Brain, skeletal muscle and platelet mitochondrial function in Parkinson's disease. *Brain*, *115*, 333–342.
- Manta, B., Comini, M., Medeiros, A., Hugo, M., Trujillo, M., & Radi, R. (2013). Trypanothione: a unique bis-glutathionyl derivative in trypanosomatids. *Biochimica et Biophysica Acta*, *1830*, 3199–3216.
- Marchand, D., & Abdel-Monem, M. M. (1985). Glutathione *S*-transferases catalyzed conjugation of 1,4-disubstituted butanes with glutathione in vitro. *Biochemical and Biophysical Research Communications*, *128*, 360–367.
- Marchand, D. H., Rimmel, R. P., & Abdel-Monem, M. M. (1988). Biliary excretion of a glutathione conjugate of busulfan and 1,4-diodobutane in the rat. *Drug Metabolism and Disposition*, *16*, 85–92.
- Marjot, R., & McLeod, A. A. (1989). Chronic non-neurological toxicity from volatile substance abuse. *Human Toxicology*, *8*, 301–306.
- Mårtensson, J., Lai, J. C. K., & Meister, A. (1990). High-affinity transport of glutathione is part of a multicomponent system essential for mitochondrial function. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 7185–7189.
- Martin, J. L., George, J. W., & Pohl, L. R. (1980a). Glutathione-dependent dechlorination of chloramphenicol by cytosol of rat liver. *Drug Metabolism and Disposition*, *8*, 93–97.

- Martin, J. L., Gross, B. J., Morris, P., & Pohl, L. R. (1980b). Mechanism of glutathione-dependent dechlorination of chloramphenicol and thiamphenicol by cytosol of rat liver. *Drug Metabolism and Disposition*, *8*, 371–375.
- Martin, M. N., Saladores, P. H., Lambert, E., Hudson, A. O., & Leustek, T. (2007). Localization of members of the γ -glutamyl transpeptidase family identifies sites of glutathione and glutathione S-conjugate hydrolysis. *Plant Physiology*, *144*, 1715–1732.
- Mayatepek, E., Okun, J. G., Meissner, T., Assmann, B., Hammond, J., Zschocke, J., & Lehmann, W. D. (2004). Synthesis and metabolism of leukotrienes in gamma-glutamyl transpeptidase deficiency. *Journal of Lipid Research*, *45*, 900–904.
- McCartney, R. G., Rice, J. E., Sanderson, S. J., Bunik, V., Lindsay, H., & Lindsay, J. G. (1998). Subunit interactions in the mammalian α -ketoglutarate dehydrogenase complex. Evidence for direct association of the α -ketoglutarate dehydrogenase and dihydroliipoamide dehydrogenase components. *Journal of Biological Chemistry*, *273*, 24158–24164.
- McGoldrick, T. A., Lock, E. A., Rodilla, V., & Hawsworth, G. M. (2003). Renal conjugate C-S lyase mediated toxicity of halogenated alkenes in primary cultures of human and rat proximal tubular cells. *Archives of Toxicology*, *77*, 365–370.
- Mehta, P. K., & Christen, P. (2000). The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes. *Advances in Enzymology and Related Areas of Molecular Biology*, *74*, 129–184.
- Meister, A., Sober, H. A., Tice, S. V., & Fraser, P. E. (1952). Transamination and associated deamidation of asparagine and glutamine. *Journal of Biological Chemistry*, *197*, 319–330.
- Menon, D., & Board, P. G. (2013). A role for glutathione transferase Omega 1 (GSTO1-1) in the glutathionylation cycle. *Journal of Biological Chemistry*, *288*, 25769–25779.
- Metzler, D. E. (2001). In *Biochemistry: The Chemical Reactions of Living Cells* (vol. 1, pp. 737–753). San Diego, CA: Harcourt/Academic Press.
- Miller, J. E., & Litwack, G. (1971). Purification, properties, and identity of liver mitochondrial tyrosine aminotransferase. *Journal of Biological Chemistry*, *246*, 3234–3240.
- Mishra, P. K., Raghuram, G. V., Panwar, H., Jain, D., Pandey, H., & Maudar, K. K. (2009). Mitochondrial oxidative stress elicits chromosomal instability after exposure to isocyanates in human kidney epithelial cells. *Free Radical Research*, *43*, 718–728.
- Mishra, P. K., Raghuram, G. V., Bunkar, N., Bhargava, A., & Khare, N. K. (2015). Molecular bio-dosimetry for carcinogenic risk assessment in survivors of Bhopal gas tragedy. *International Journal of Occupational Medicine and Environmental Health*, *28*, 921–939.
- Mitchell, S. C., Smith, R. L., Waring, R. H., & Aldington, G. F. (1984). The metabolism of S-methyl-L-cysteine in man. *Xenobiotica*, *14*, 767–779.
- Mitta, M., Ohnogi, H., Yamamoto, A., Kato, I., Sakiyama, F., & Tsunasawa, S. (1992). The primary structure of porcine aminoacylase I deduced from cDNA sequence. *Journal of Biochemistry*, *112*, 737–742.
- Mitta, M., Kato, I., & Tsunasawa, S. (1993). The nucleotide sequence of human aminoacylase-1. *Biochimica et Biophysica Acta*, *1174*, 201–203.
- Mizuno, Y., Ohta, S., Tanaka, M., Takamiya, S., Suzuki, K., Sato, T., Oya, H., Ozawa, T., & Kagawa, Y. (1989). Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. *Biochemical and Biophysical Research Communications*, *163*, 1450–1455.
- Mizuno, Y., Matuda, S., Yoshino, H., Mori, H., Hattori, N., & Ikebe, S. (1994). An immunohistochemical study on α -ketoglutarate dehydrogenase complex in Parkinson's disease. *Annals of Neurology*, *35*, 204–210.
- Monks, T. J., & Lau, S. S. (1994). Glutathione conjugation as a mechanism for the transport of reactive metabolites. *Advances in Pharmacology*, *27*, 183–210.
- Monks, T. J., Lau, S. S., Highet, R. J., & Gillette, J. R. (1985). Glutathione conjugates of 2-bromohydroquinone are nephrotoxic. *Drug Metabolism and Disposition*, *13*, 553–559.
- Montine, T. J., Amarnath, V., Picklo, M. J., Sidell, K. R., Zhang, J., & Graham, D. G. (2000). Dopamine mercapturate can augment dopaminergic neurodegeneration. *Drug Metabolism Reviews*, *32*, 363–376.
- Moore, K. P., Taylor, G. W., Gove, C., Wood, J., Tan, K. L., Eason, J., & William, R. (1992). Synthesis and metabolism of cysteinyl leukotrienes by the isolated pig kidney. *Kidney International*, *41*, 1543–1548.
- Morino, Y., Osman, A. M., & Okamoto, M. (1974). Formate-induced labeling of the active site of aspartate aminotransferase by β -chloro-L-alanine. *Journal of Biological Chemistry*, *249*, 6684–6692.
- Morrow, A. L., Williams, K., Sand, A., Boanca, G., & Barycki, J. J. (2007). Characterization of *Helicobacter pylori* γ -glutamyltranspeptidase reveals the molecular basis for substrate specificity and a critical role for the tyrosine 433-containing loop in catalysis. *Biochemistry*, *46*, 13407–13414.
- Mosca, M., Cozzi, L., Breton, J., Speciale, C., Okuno, E., Schwarcz, R., & Benatti, L. (1994). Molecular cloning of rat kynurenine aminotransferase: identity with glutamine transaminase K. *FEBS Letters*, *353*, 21–24.
- Mosca, M., Croci, C., Mostardini, M., Breton, J., Malyszko, J., Avanzi, N., Toma, S., Benatti, L., & Gatti, S. (2003). Tissue expression and translational control of rat kynurenine aminotransferase/glutamine transaminase K mRNAs. *Biochimica et Biophysica Acta*, *1628*, 1–10.
- Müller, M., Birner, G., Sander, M., & Dekant, W. (1998). Reactivity of haloalkenes with nucleobases: reactions in vitro with DNA. *Chemical Research in Toxicology*, *11*, 464–470.
- Murphy, R. C., & Gijón, M. A. (2007). Biosynthesis and metabolism of leukotrienes. *Biochemical Journal*, *405*, 379–395.
- Nachtomi, E. (1970). The metabolism of ethylene dibromide in the rat: the enzymatic reaction with glutathione in vitro and in vivo. *Biochemical Pharmacology*, *19*, 2853–2860.
- Naim, H. Y., Joberty, G., Alfalah, M., & Jacob, R. (1999). Temporal association of the N- and O-linked glycosylation events and their implication in the polarized sorting of intestinal brush border sucrose-isomaltase, aminopeptidase N, and dipeptidyl peptidase IV. *Journal of Biological Chemistry*, *274*, 17961–17967.
- Nakajima, M., Watanabe, B., Han, L., Shimizu, B., Wada, K., Fukuyama, K., Suzuki, H., & Hiratake, J. (2014). Glutathione-analogous peptidyl phosphorus esters as mechanism-based inhibitors of gamma-glutamyl transpeptidase for probing cysteinyl-glycine binding site. *Bioorganic and Medicinal Chemistry*, *22*, 1176–1194.
- Nash, B., & Tate, S. S. (1982). Biosynthesis of rat renal γ -glutamyl transpeptidase. *Journal of Biological Chemistry*, *257*, 585–588.
- Nash, J. A., King, L. J., Lock, E. A., & Green, T. (1984). The metabolism and disposition of hexachloro-1,3-butadiene in the rat and its relevance to nephrotoxicity. *Toxicology and Applied Pharmacology*, *73*, 124–137.
- Natsch, A., Schmid, J., & Flachsmann, F. (2004). Identification of odiferous sulfanylalkanols in human axilla secretions and their formation through cleavage of cysteine precursors by a C-S lyase isolated from axilla bacteria. *Chemistry and Biodiversity*, *1*, 1058–1072.
- Needleman, P., Blehm, D. J., & Rotskoff, K. S. (1969). Relationship between glutathione-dependent denitration and the vasodilator effects of organic nitrates. *Journal of Pharmacology and Experimental Therapeutics*, *165*, 286–288.
- Newman, D., Abuladze, N., Scholz, K., Dekant, W., Tsuprun, V., Ryazantsev, S., Bondar, G., Sassani, P., Kurtz, I., & Pushkin, A. (2007). Specificity of aminoacylase II-mediated deacetylation of mercapturic acids. *Drug Metabolism and Disposition*, *35*, 43–50.
- Niehaus, T. D., Nguyen, T. N., Gidda, S. K., ElBadawi-Sidhu, M., Lambrecht, J. A., McCarty, D. R., Downs, D. M., Cooper, A. J. L., Fiehn, O., Mullen, R. T., & Hanson, A. D. (2014). *Arabidopsis* and maize RdA proteins preempt reactive enamine/imine damage to branched-chain amino acid biosynthesis in plastids. *Plant Cell*, *26*, 3010–3022.
- Niehaus, T. D., Gerdes, S., Hodge-Hanson, K., Zhukov, A., Cooper, A. J. L., ElBadawi-Sidhu, M., Fiehn, O., Downs, D. M., & Hanson, A. D. (2015). Genomic and experimental evidence for multiple metabolic functions in the RdA/YjgF/YER057c/UK114 (Rid) protein family. *BMC Genomics*, *16*, 382.
- Nies, A. T., & Keppler, D. (2007). The apical conjugate efflux pump ABC2 (MRP2). *Pflügers Archiv: European Journal of Physiology*, *453*, 643–659.
- Odum, J., & Green, T. (1984). The metabolism and nephrotoxicity of tetrafluoroethylene in the rat. *Toxicology and Applied Pharmacology*, *76*, 306–318.
- Okada, T., Suzuki, H., Wada, K., Kumagai, H., & Fukuyama, K. (2006). Crystal structures of γ -glutamyltranspeptidase from *Escherichia coli*, a key enzyme in glutathione metabolism, and its reaction intermediate. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 6471–6476.
- Okajima, K., Inoue, M., Morino, Y., & Itoh, K. (1984). Topological aspects of microsomal N-acetyltransferase, an enzyme responsible for the acetylation of cysteine S-conjugates of xenobiotics. *European Journal of Biochemistry*, *142*, 281–286.
- Olsen, J., Cowell, G. M., Køningshøfer, E., Danielsen, E. M., Møller, J., Laustsen, L., Hansen, O. C., Welinder, K. G., Engberg, J., Hunziker, W., Spiesst, M., Sjöström, H., & Norén, O. (1988). Complete amino acid sequence of human intestinal aminopeptidase N as deduced from the cloned cDNA. *FEBS Letters*, *238*, 307–314.
- Onkenhout, W., van Loon, W. M., Buijs, W., van der Gen, A., & Vermeulen, N. P. E. (1986). Biotransformation and quantitative determination of sulfur-containing metabolites of 1,4-dibromobutane in the rat. *Drug Metabolism and Disposition*, *14*, 608–612.

- Orlowski, M., & Meister, A. (1963). γ -Glutamyl-*p*-nitroanilide: a new convenient substrate for determination and study of L- and D- γ -glutamyltranspeptidase activities. *Biochimica et Biophysica Acta*, *73*, 679–681.
- Orlowski, M., & Meister, A. (1965). Isolation of γ -glutamyl transpeptidase from hog kidney. *Journal of Biological Chemistry*, *240*, 338–347.
- Orlowski, M., & Meister, A. (1970). The γ -glutamyl cycle: a possible transport system for amino acids. *Proceedings of the National Academy of Sciences of the United States of America*, *67*, 1248–1255.
- Örning, L., Norin, E., Gustafsson, B., & Hammerström, S. (1986). In vivo metabolism of leukotriene C₄ in germ-free and conventional rats. Fecal excretion of N-acetylleukotriene E₄. *Journal of Biological Chemistry*, *261*, 766–771.
- Ovádi, J., & Srere, P. A. (2000). Macromolecular compartmentation and channeling. *International Review of Cytology*, *192*, 255–280.
- Pandur, S., Pankiv, S., Johannessen, M., Moens, U., & Huseby, N. E. (2007). γ -Glutamyltransferase is upregulated after oxidative stress through the Ras signal transduction pathway in rat colon carcinoma cells. *Free Radical Research*, *41*, 1376–1384.
- Park, S. B., Osterloh, J. D., Vamvakas, S., Hashmi, M., Anders, M. W., & Cashman, J. R. (1992). Flavin-containing monooxygenase-dependent stereoselective S-oxygenation and cytotoxicity of cysteine S-conjugates and mercapturates. *Chemical Research in Toxicology*, *5*, 193–201.
- Park, L. C. H., Gibson, G. E., Bunik, V., & Cooper, A. J. L. (1999). Inhibition of select mitochondrial enzymes in PC12 cells exposed to S-(1,1,2,2-tetrafluoroethyl)-L-cysteine. *Biochemical Pharmacology*, *58*, 1557–1565.
- Parl, J. A., Godfrey, D. A., & Ross, C. D. (1987). Separate enzymatic microassays for aspartate aminotransferase isoenzymes. *Biochimica et Biophysica Acta*, *925*, 175–184.
- Perrier, J., Durand, A., Giardina, T., & Puigserver, A. (2004). The rat kidney acylase 1. Evidence for a new cDNA form and comparisons with the porcine intestinal enzyme. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, *138*, 277–283.
- Perry, S. J., Schofield, M. A., MacFarlane, M., Lock, E. A., King, L. J., Gibson, G. G., & Goldfarb, P. S. (1993). Isolation and expression of a cDNA coding for rat kidney cytosolic cysteine conjugate β -lyase. *Molecular Pharmacology*, *43*, 660–665.
- Perry, S., Harries, H., Schofield, C., Lock, T., King, L., Gibson, G., & Goldfarb, P. (1995). Molecular cloning and expression of a cDNA for human kidney cysteine conjugate β -lyase. *FEBS Letters*, *360*, 277–280.
- Pfaller, W., & Gstraunthaler, G. (1998). Nephrotoxicity testing in vitro—what we know and what we need to know. *Environmental Health Perspectives*, *106*(Suppl. 2), 559–569.
- Pinto, J. T., Krasnikov, B. F., & Cooper, A. J. L. (2006). Redox-sensitive proteins are potential targets of garlic-derived mercaptocysteine derivatives. *Journal of Nutrition*, *136*, S835–S841.
- Pinto, J. T., Krasnikov, B. F., Alcutt, S., Jones, M. E., Dorai, T., Villar, M. T., Artigues, A., Li, J., & Cooper, A. J. L. (2014). Kynurenine aminotransferase III and glutamine transaminase L are identical enzymes that have cysteine S-conjugate β -lyase activity and can transaminate L-selenomethionine. *Journal of Biological Chemistry*, *289*, 30950–30961.
- Pohl, L. R., Branchflower, R. V., Highet, R. J., Martin, J. L., Nunn, D. S., Monks, T. J., George, J. W., & Hinson, J. A. (1981). The formation of glutathionyl dithiocarbonate as a metabolite of chloroform, bromotrichloromethane, and carbon tetrachloride. *Drug Metabolism and Disposition*, *9*, 334–339.
- Pombrio, J. M., Giangreco, A., Li, L., Wempe, M. F., Anders, M. W., Sweet, D. H., Pritchard, J. B., & Ballatori, N. (2001). Mercapturic acids (N-acetylcysteine S-conjugates) as endogenous substrates of the renal organic anion transporter-1. *Molecular Pharmacology*, *60*, 1091–1099.
- Pompella, A., De Tata, V., Paolicchi, A., & Zunino, F. (2006). Expression of γ -glutamyltransferase in cancer cells and its significance in drug resistance. *Biochemical Pharmacology*, *71*, 231–238.
- Poon, J. C., & Joseph, P. D. (2012). Hydrolysis of S-aryl-cysteinyglycine conjugates catalyzed by porcine kidney cortex membrane dipeptidase. *Xenobiotica*, *42*, 1178–1186.
- Potdar, P. D., Andrews, K. L., Nettesheim, P., & Ostrowski, L. E. (1997). Expression and regulation of γ -glutamyl transpeptidase-related enzyme in tracheal cells. *American Journal of Physiology*, *273*, L1082–L1089.
- Rajpert-De Meyts, E., Heisterkamp, N., & Groffen, J. (1988). Cloning and nucleotide sequence of human γ -glutamyl transpeptidase. *Proceedings of the National Academy of Sciences of the United States of America*, *85*, 8840–8844.
- Raphel, V., Giardina, T., Guevel, L., Perrier, J., Dupuis, L., Guo, X. J., & Puigserver, A. (1999). Cloning, sequencing and further characterization of acylpeptidase hydrolase from porcine intestinal mucosa. *Biochimica et Biophysica Acta*, *1432*, 371–381.
- Rebbeor, J. F., Wang, W., Clifton, D., & Ballatori, N. (1998). Glutathione S-conjugate formation and metabolism in HepG2 cells: a cell model of mercapturic acid biosynthesis. *Journal of Toxicology and Environmental Health A*, *53*, 651–663.
- Reichert, D., Liebaltd, G., & Henschler, D. (1976). Neurotoxic effects of dichloroacetylene. *Archives of Toxicology*, *37*, 23–38.
- Reuter, S., Schnekenburger, M., Cristofanon, S., Buck, I., Teiten, M. H., Daubeuf, S., Eifes, S., Dicato, M., Aggarwal, B. B., Visvikis, A., & Diederich, M. (2009). Tumor necrosis factor α induces γ -glutamyltransferase expression via nuclear factor-kappaB in cooperation with Sp1. *Biochemical Pharmacology*, *77*, 397–411.
- Riccioni, G., Bucciarelli, T., Mancini, B., Di Ilio, C., & D'Orazio, N. (2007). Antileukotriene drugs: clinical application, effectiveness and safety. *Current Medicinal Chemistry*, *14*, 1966–1977.
- Ripp, S. L., Overby, L. H., Philpot, R. M., & Elfarra, A. (1997). Oxidation of cysteine S-conjugates by rabbit liver microsomes and cDNA-expressed flavin-containing monooxygenases: studies with S-(1,2-dichlorovinyl)-L-cysteine, S-(1,2,2-trichlorovinyl)-L-cysteine, S-allyl-L-cysteine, and S-benzyl-L-cysteine. *Molecular Pharmacology*, *51*, 507–515.
- Ritter, C. A., Bohnenstengel, F., Hofmann, U., Kroemer, H. K., & Sperker, B. (1999). Determination of tetrahydrothiophene formation as a probe of in vitro busulfan metabolism by human glutathione S-transferase A1-1: use of a highly sensitive gas chromatographic-mass spectrometric method. *Journal of Chromatography. B, Biomedical Sciences and Applications*, *730*, 25–31.
- Ritter, C. A., Sperker, B., Grube, M., Dressel, D., Kunert-Keil, C., & Kroemer, H. K. (2002). Overexpression of glutathione S-transferase A1-1 in ECV 304 cells protects against busulfan mediated G2-arrest and induces tissue factor expression. *British Journal of Pharmacology*, *137*, 1100–1106.
- Roberts, J. J., & Warwick, G. P. (1961). The mode of action of alkylating agents. III. The formation of 3-hydroxytetrahydrothiophene-1:1-dioxide from 1:4-dimethanesulphonyloxybutane (myleran), S- β -L-alanyl-tetrahydrothiophenium mesylate, tetrahydrothiophene and tetrahydrothiophene-1:1-dioxide in the rat, rabbit and mouse. *Biochemical Pharmacology*, *6*, 217–227.
- Rosenberg, M. F., Oleschuk, C. J., Wu, P., Mao, Q., Deeley, R. G., Cole, S. P., & Ford, R. C. (2010). Structure of a human multidrug transporter in an inward-facing conformation. *Journal of Structural Biology*, *170*, 540–547.
- Rosner, E., Müller, M., & Dekant, W. (1998). Stereo- and regioselective conjugation of S-halovinyl mercapturic acid sulfoxides by glutathione S-transferases. *Chemical Research in Toxicology*, *11*, 12–18.
- Rossi, F., Han, Q., Li, J., Li, J., & Rizzi, M. (2004). Crystal structure of human kynurenine aminotransferase I. *Journal of Biological Chemistry*, *279*, 50214–50220.
- Rossi, M., Bolz, C., Revez, J., Javed, S., El-Najjar, N., Anderl, F., Hyttiäinen, H., Vuorela, P., Gerhard, M., & Hanninen, M. L. (2012). Evidence for conserved function of gamma-glutamyltranspeptidase in Helicobacter genus. *PLoS One*, *7*, e30543.
- Rubio-Allaga, I., & Daniel, H. (2002). Mammalian peptide transporters as targets for drug delivery. *Trends in Pharmacological Sciences*, *23*, 434–440.
- Rusyn, I., Chiu, W. A., Lash, L. H., Kromhout, H., Hansen, J., & Guyton, K. Z. (2014). Trichloroethylene: mechanistic, epidemiologic and other supporting evidence of carcinogenic hazard. *Pharmacology and Therapeutics*, *141*, 55–68.
- Ryazantsev, S., Abuladze, N., Newman, D., Bondar, G., Kurtz, I., & Pushkin, A. (2007). Structural characterization of dimeric murine aminoacylase III. *FEBS Letters*, *581*, 1898–1902.
- Saari, J. C., & Schultze, M. O. (1965). Cleavage of S-(1,2-dichlorovinyl)-L-cysteine by *Escherichia coli* B. *Archives of Biochemistry and Biophysics*, *109*, 595–602.
- Sakamuro, D., Yamazoe, M., Matsuda, Y., Kangawa, K., Taniguchi, N., Matsuo, H., Yoshikawa, H., & Ogasawara, N. (1988). The primary structure of human γ -glutamyl transpeptidase. *Gene*, *73*, 1–9.
- Samarth, R. M., Gandhi, P., & Maudar, K. K. (2013). A retrospective review of cytogenetic studies on methyl isocyanate with special reference to the Bhopal gas tragedy: is the next generation also at risk? *International Journal of Occupational Medicine and Environmental Health*, *26*, 324–336.

- Sausen, P. J., & Elfarrar, A. A. (1991). Reactivity of cysteine S-conjugate sulfoxides: formation of S-[1-chloro-2-(S-glutathionyl)vinyl]-L-cysteine sulfoxide by the reaction of S-(1,2-dichlorovinyl)-L-cysteine sulfoxide with glutathione. *Chemical Research in Toxicology*, *4*, 655–660.
- Sausen, P. J., Duescher, R. J., & Elfarrar, A. A. (1993). Further characterization and purification of the flavin-dependent S-benzyl-L-cysteine S-oxidase activities of rat liver and kidney. *Molecular Pharmacology*, *43*, 388–396.
- Schaeffer, V. H., & Stevens, J. L. (1987a). The transport of S-cysteine conjugates in LLC-PK₁ cells and its role in toxicity. *Molecular Pharmacology*, *31*, 506–512.
- Schaeffer, V. H., & Stevens, J. L. (1987b). Mechanism of transport for toxic cysteine conjugates in rat kidney cortex membrane vesicles. *Molecular Pharmacology*, *32*, 293–298.
- Schapiro, A. H. V., Mann, V. M., Cooper, J. M., Dexter, D., Daniel, S. E., Jenner, P., Clark, J. B., & Marsden, C. D. (1990). Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *Journal of Neurochemistry*, *55*, 2142–2145.
- Schaub, T., Ishikawa, T., & Keppler, D. (1991). ATP-Dependent leukotriene export from mastocytoma cells. *FEBS Letters*, *279*, 83–86.
- Schlosser, P. M., Bale, A. S., Gibbons, C. F., Wilkins, A., & Cooper, G. S. (2015). Human health effects of dichloromethane: key findings and scientific issues. *Environmental Health Perspectives*, *123*, 114–119.
- Schneider, G., Käck, H., & Lindqvist, Y. (2000). The manifold of vitamin B₆ dependent enzymes. *Structure*, *8*, R1–R6.
- Schrenk, D., Dekant, W., Wünsch, P. H., & Henschler, D. (1988a). Role of metabolic activation in the toxicity of S-(pentachlorobutadienyl)glutathione and S-(pentachlorobutadienyl)-L-cysteine in the isolated perfused rat kidney. *Toxicology In Vitro*, *2*, 283–290.
- Schrenk, D., Dekant, W., & Henschler, D. (1988b). Metabolism and excretion of S-conjugates derived from hexachlorobutadiene in the isolated perfused rat kidney. *Molecular Pharmacology*, *34*, 407–412.
- Scian, M., & Atkins, W. M. (2015). The busulfan metabolite EdAG irreversibly glutathionylates glutaredoxins. *Archives of Biochemistry and Biophysics*, *583*, 96–104.
- Sekioka, T., Kadode, M., Fujii, M., Kawabata, K., Abe, T., Horiba, M., Kohno, S., & Nabe, T. (2015). Expression of CysLT2 receptors in asthma lung, and their possible role in bronchoconstriction. *Allergy International*, *64*, 351–358.
- Shen, X.-M., Xia, B., Wrona, M. Z., & Dryhurst, G. (1996). Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteine conjugates of dopamine: possible metabolites of relevance to Parkinson's disease. *Chemical Research in Toxicology*, *9*, 1117–1126.
- Shen, X.-M., Zhang, F., & Dryhurst, G. (1997). Oxidation of dopamine in the presence of L-cysteine: characterization of new toxic products. *Chemical Research in Toxicology*, *10*, 147–155.
- Shen, X.-M., Li, H., & Dryhurst, G. (2000). Oxidative metabolites of 5-S-cysteinyl-dopamine inhibit the α -ketoglutarate dehydrogenase complex: possible relevance to the pathogenesis of Parkinson's disease. *Journal of Neural Transmission*, *107*, 959–978.
- Shimomura, N., Honma, M., Chiba, S., Tahara, S., & Mizutani, J. (1992). Cysteine-conjugate β -lyase from *Mucor javanicus*. *Bioscience, Biotechnology, and Biochemistry*, *56*, 963–964.
- Shrawder, E., & Martinez-Carrion, M. (1972). Evidence of phenylalanine transaminase activity in the isozymes of aspartate aminotransferase. *Journal of Biological Chemistry*, *247*, 2486–2492.
- Sibhatu, M. B., Smitherman, P. K., Townsend, A. J., & Morrow, C. S. (2008). Expression of MRP1 and GST1-1 modulate the acute cellular response to treatment with the chemopreventive isothiocyanate, sulforaphane. *Carcinogenesis*, *29*, 807–815.
- Silbernagl, S., & Heuner, A. (1993). Renal transport of mercapturic acids and their precursors, the S-conjugates of glutathione and cysteine. In M. W. Anders, W. Dekant, D. Henschler, H. Oberleithner, & S. Silbernagl (Eds.), *Renal Disposition and Nephrotoxicity of Xenobiotics* (pp. 135–154). San Diego, CA: Academic Press, Inc.
- Silva, M. S., Barata, L., Ferreira, A. E., Romão, S., Tomás, A. M., Freire, A. P., & Cordeiro, C. (2008). Catalysis and structural properties of *Leishmania infantum* glyoxalase II: trypanothione specificity and phylogeny. *Biochemistry*, *47*, 195–204.
- Silverman, R. B. (1992). *Organic Chemistry of Drug Design and Drug Chemistry*. New York, NY: Academic Press. pp. 336–338.
- Singh, R. K., Gupta, S., Dastidar, S., & Ray, A. (2010). Cysteinyl leukotrienes and their receptors: molecular and functional characteristics. *Pharmacology*, *85*, 336–349.
- Sklan, N. M., & Barnsley, E. A. (1968). The metabolism of S-methyl-L-cysteine. *Biochemical Journal*, *107*, 217–223.
- Slot, A. J., Wise, D. D., Deeley, R. G., Monks, T. J., & Cole, S. P. C. (2008). Modulation of human multidrug resistance protein (MRP)1 (ABCC1) and MRP2 (ABCC2) transport activities by endogenous and exogenous glutathione-conjugated catechol metabolites. *Drug Metabolism and Disposition*, *36*, 552–560.
- Söderström, M., Hammarström, S., & Mannervik, B. (1988). Leukotriene C synthase in mouse mastocytoma cells. An enzyme distinct from cytosolic and microsomal glutathione transferases. *Biochemical Journal*, *250*, 713–718.
- Spencer, J. P. E., Jenner, P., & Halliwell, B. (1995). Superoxide-dependent depletion of reduced glutathione by L-DOPA and dopamine. Relevance to Parkinson's disease. *NeuroReport*, *6*, 1480–1484.
- Spencer, J. P. E., Jenner, P., Daniel, S. E., Lees, A. J., Marsden, D. C., & Halliwell, B. (1998). Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. *Journal of Neurochemistry*, *71*, 2112–2122.
- Spencer, J. P. E., Whiteman, M., Jenner, P., & Halliwell, B. (2002). 5-S-Cysteinyl-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases in caspase-3 activity in neurons. *Journal of Neurochemistry*, *81*, 122–129.
- Starkenmann, C., & Niclass, Y. (2011). New cysteine-S-conjugate precursors of volatile sulfur compounds in bell peppers (*Capsicum annuum* L. cultivar). *Journal of Agricultural and Food Chemistry*, *59*, 3358–3365.
- Starkenmann, C., Niclass, Y., & Escher, S. (2007). Volatile organic sulfur-containing constituents in *Poncirus trifoliata* (L.) Raf. (Rutaceae). *Journal of Agricultural and Food Chemistry*, *55*, 4511–4517.
- Starkenmann, C., Le Calvé, B., Niclass, Y., Cayeux, I., Beccucci, S., & Troccaz, M. (2008). Olfactory perception of cysteine-S-conjugates from fruits and vegetables. *Journal of Agricultural and Food Chemistry*, *56*, 9575–9580.
- Starkenmann, C., Niclass, Y., & Troccaz, M. (2011). Nonvolatile S-alk(enyl)thio-L-cysteine derivatives in fresh onion (*Allium cepa* L. cultivar). *Journal of Agricultural and Food Chemistry*, *59*, 9457–9465.
- Stevens, J. L. (1985). Isolation and characterization of a rat liver enzyme with both cysteine conjugate β -lyase and kynureninase activity. *Journal of Biological Chemistry*, *260*, 7945–7950.
- Stevens, J. L., & Anders, M. W. (1979). Metabolism of haloforms to carbon monoxide. III. Studies on the mechanism of the reaction. *Biochemical Pharmacology*, *28*, 3189–3194.
- Stevens, J. L., & Anders, M. W. (1981). Metabolism of haloforms to carbon monoxide. IV. Studies on the mechanism in vivo. *Chemico-Biological Interactions*, *37*, 365–374.
- Stevens, J. L., & Jones, D. P. (1989). The mercapturic acid pathway: biosynthesis, intermediary metabolism, and physiological disposition. In D. Dolphin, R. Poulson, & O. Avramović (Eds.), *Glutathione. Chemical, Biochemical and Medicinal Aspects, Part B* (pp. 45–84). New York, NY: Wiley.
- Stevens, J. L., Robbins, J. D., & Byrd, R. A. (1986). A purified cysteine conjugate β -lyase from rat kidney cytosol. Requirement for an α -keto acid or an amino acid oxidase for activity and identity with soluble glutamine transaminase K. *Journal of Biological Chemistry*, *261*, 15529–15537.
- Suzuki, S., Tomisawa, H., Ichihara, S., & Fukuzawa, M. (1982). A C-S bond cleavage enzyme of cysteine conjugate in intestinal microorganisms. *Biochemical Pharmacology*, *31*, 2137–2140.
- Szewczuk, A., & Baranowski, T. (1963). Purification and properties of γ -glutamyl transpeptidase from beef kidney. *Biochemische Zeitschrift*, *338*, 317–329.
- Taniguchi, N., & Ikeda, Y. (1998). γ -Glutamyl transpeptidase: catalytic mechanism and gene expression. *Advances in Enzymology and Related Areas of Molecular Biology*, *72*, 239–278.
- Taniguchi, N., Higashi, T., Sakamoto, Y., & Meister, A. (Eds.). (1989). *Glutathione Centennial: Molecular Perspectives and Clinical Implications*. Chichester: Wiley.
- Tate, S. S. (1985). Microvillus membrane peptidases that catalyze hydrolysis of cysteinylglycine and its derivatives. *Methods in Enzymology*, *113*, 471–484.
- Tate, S. S. (1989). Glutathione-degrading enzymes of brush border: molecular and functional aspects. In N. Taniguchi, T. Higashi, Y. Sakamoto, & A. Meister (Eds.), *Glutathione Centennial: Molecular Perspectives and Clinical Implications* (pp. 189–208). Chichester: Wiley.
- Tate, S. S., & Meister, A. (1978). Serine-borate complex as a transition-state inhibitor of γ -glutamyl transpeptidase. *Proceedings of the National Academy of Sciences of the United States of America*, *75*, 4806–4809.
- Tate, S. S., & Meister, A. (1985). γ -Glutamyl transpeptidase from kidney. *Methods in Enzymology*, *113*, 400–419.

- Tateishi, M., Suzuki, S., & Shimizu, H. (1978a). The metabolism of bromazepam in the rat—identification of mercapturic acid and its conversion in vitro to methylthio-bromazepam. *Biochemical Pharmacology*, *27*, 809–810.
- Tateishi, M., Suzuki, S., & Shimizu, H. (1978b). Cysteine conjugate β -lyase in rat liver. A novel enzyme catalyzing formation of thiol-containing metabolites of drugs. *Journal of Biological Chemistry*, *253*, 8854–8859.
- Teichert, J., Sohr, R., Hennig, L., Baumann, F., Schoppmeyer, K., Patzak, U., & Preiss, R. (2009). Identification and quantitation of the *N*-acetyl-L-cysteine *S*-conjugates of bendamustine and its sulfoxides in human bile after administration of bendamustine hydrochloride. *Drug Metabolism and Disposition*, *37*, 292–301.
- Terzyan, S. S., Burgett, A. W., Heroux, A., Smith, C. A., Mooers, B. H., & Hanigan, M. H. (2015). Human gamma-glutamyl transpeptidase 1: structures of the free enzyme, inhibitor-bound tetrahedral transition states, and glutamate-bound enzyme reveal novel movement within the active site during catalysis. *Journal of Biological Chemistry*, *290*, 17576–17586.
- Tew, K. D., & Townsend, D. M. (2012). Glutathione-*S*-transferases as determinants of cell survival and death. *Antioxidants and Redox Signaling*, *17*, 1728–1737.
- Thompson, G. A., & Meister, A. (1977). Interrelationships between the binding sites for amino acids, dipeptides, and γ -glutamyl donors in γ -glutamyl transpeptidase. *Journal of Biological Chemistry*, *252*, 6792–6798.
- Tomisawa, H., Suzuki, S., Ichihara, S., Fukazawa, H., & Tateishi, M. (1984). Purification and characterization of a C-S lyase from *Fusobacterium varium*. A C-S cleavage enzyme of cysteine conjugates and some S-containing amino acids. *Journal of Biological Chemistry*, *259*, 2588–2593.
- Tomisawa, H., Ichihara, S., Fukazawa, H., Ichimoto, N., Tateishi, M., & Yamamoto, I. (1986). Purification and characterization of human hepatic cysteine-conjugate β -lyase. *Biochemical Journal*, *235*, 569–575.
- Tomisawa, H., Ichimoto, N., Ichihara, S., Fukazawa, H., & Tateishi, M. (1988). Involvement of cystathionase in the formation of alkane-thiols from corresponding cysteine conjugates. *Xenobiotica*, *18*, 1029–1037.
- Townsend, D. M., Lushchak, V. I., & Cooper, A. J. L. (2014). A comparison of reversible versus irreversible protein glutathionylation. *Advances in Cancer Research*, *122*, 177–198.
- Tsao, B., & Curthoys, N. P. (1980). The absolute asymmetry of orientation of γ -glutamyltranspeptidase and aminopeptidase on the external surface of the rat renal brush border membrane. *Journal of Biological Chemistry*, *255*, 7708–7711.
- Tsirulnikov, K., Abuladze, N., Bragin, A., Faull, K., Cascio, D., Darnoiseaux, R., Schibler, M. J., & Pushkin, A. (2012). Inhibition of aminoacylase 3 protects rat brain cortex neuronal cells from the toxicity of 4-hydroxy-2-nonenal mercapturate and 4-hydroxy-2-nonenal. *Toxicology and Applied Pharmacology*, *263*, 303–314.
- Turnbull, L. B., Teng, L., Kinzie, J. M., Pitts, J. E., Pinchbeck, F. M., & Bruce, R. B. (1978). Excretion and biotransformation of carboxymethyl-cysteine in rat, dog, monkey and man. *Xenobiotica*, *8*, 621–628.
- Tuzova, M., Jean, J. C., Hughey, R. P., Brown, L. A., Cruikshank, W. W., Hiratake, J., & Joyce-Brady, M. (2014). Inhibiting lung lining fluid glutathione metabolism with GGsTop as a novel treatment for asthma. *Frontiers in Pharmacology*, *5*, 179.
- Ubiyovok, V. M., Blazhenko, O. V., Gigot, D., Penninckx, M., & Sibirny, A. A. (2006). Role of γ -glutamyltranspeptidase in detoxification of xenobiotics in the yeasts *Hansenula polymorpha* and *Saccharomyces cerevisiae*. *Cell Biology International*, *30*, 665–671.
- Ubuka, T., Umemura, S., Yuasa, S., Kinuta, M., & Watanabe, K. (1978). Purification and characterization of mitochondrial cysteine aminotransferase from rat liver. *Physiological Chemistry and Physics*, *10*, 483–500.
- Uehara, N., Ormstad, K., Örnig, L., & Hammerström, S. (1983). Characteristics of the uptake of cysteine-containing leukotrienes by isolated hepatocytes. *Biochimica et Biophysica Acta*, *732*, 69–74.
- Ueno, H., Likos, J. J., & Metzler, D. E. (1982). Chemistry of the inactivation of cytosolic aspartate aminotransferase by serine *O*-sulfate. *Biochemistry*, *21*, 4387–4393.
- Ullrich, K. J., Rumrich, G., Wieland, T., & Dekant, W. (1989). Contraluminal *p*-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. VI. Specificity: amino acids, their *N*-methyl-, *N*-acetyl and *N*-benzoyl derivatives: Glutathione- and cysteine conjugates, di- and oligopeptides. *Pflügers Archiv*, *415*, 342–350.
- Umapathy, A., Li, B., Donaldson, P. J., & Lim, J. C. (2015). Identification and functional characterization of a GSH conjugate efflux pathway in the rat lens. *Investigative Ophthalmology & Visual Science*, *56*, 5256–5268.
- Uotila, L., & Koivusalo, M. (1997). Expression of formaldehyde dehydrogenase and *S*-formylglutathione hydrolase activities in different rat tissues. *Advances in Experimental Medicine and Biology*, *41*, 365–371.
- Uttamsingh, V., & Anders, M. W. (1999). Acylase-catalyzed deacetylation of haloalkene-derived mercapturates. *Chemical Research in Toxicology*, *12*, 937–942.
- Uttamsingh, V., Keller, D. A., & Anders, M. W. (1998a). Acylase I-catalyzed deacetylation of *N*-acetyl-L-cysteine and *S*-alkyl-*N*-acetyl-L-cysteine. *Chemical Research in Toxicology*, *11*, 800–809.
- Uttamsingh, V., Iyer, R. A., Baggs, R. B., & Anders, M. W. (1998b). Fate and toxicity of 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (compound A)-derived mercapturates in male Fischer 344 rats. *Anesthesiology*, *89*, 1174–1183.
- Uttamsingh, V., Baggs, R. B., Krenitsky, D. M., & Anders, M. W. (2000). Immunohistochemical localization of the acylases that catalyze the deacylation of *N*-acetyl-L-cysteine and haloalkene-derived mercapturates. *Drug Metabolism and Disposition*, *28*, 625–632.
- Vaidya, V. S., Shankar, K., Lock, E. A., Bucci, T. J., & Mehendale, H. M. (2003a). Role of tissue repair in survival from *S*-(1,2-dichlorovinyl)-L-cysteine-induced acute renal tubular necrosis in the mouse. *Toxicological Sciences*, *74*, 215–227.
- Vaidya, V. S., Shankar, K., Lock, E. A., Dixon, D., & Mehendale, H. M. (2003b). Molecular mechanisms of renal tissue repair in survival from acute renal tubule: role of ERK1/2 pathway. *Toxicologic Pathology*, *31*, 604–618.
- Vamvakas, S., & Köster, U. (1993). The nephrotoxin dichlorovinylcysteine induces expression of the protooncogenes *c-fos* and *c-myc* in LLC-PK₁ cells—a comparative investigation with growth factors and 12-*O*-tetradecanoylphorbolacetate. *Cell Biology and Toxicology*, *9*, 1–13.
- Vamvakas, S., Berthold, K., Dekant, W., Henschler, D., & Anders, M. W. (1988a). Bacterial cysteine conjugate β -lyase and the metabolism of cysteine *S*-conjugates: structural requirements for the cleavage of *S*-conjugates and the formation of reactive intermediates. *Chemico-Biological Interactions*, *65*, 59–71.
- Vamvakas, S., Elfarra, A. A., Dekant, W., Henschler, D., & Anders, M. W. (1988b). Mutagenicity of amino acid and glutathione *S*-conjugate in the Ames test. *Mutation Research*, *206*, 83–90.
- Vamvakas, S., Bittner, D., & Köster, U. (1993). Enhanced expression of the protooncogenes *c-myc* and *c-fos* in normal and malignant renal growth. *Toxicology Letters*, *67*, 161–172.
- Vauzour, D., Ravaioli, G., Vafeiadou, K., Rodriguez-Mateos, A., Angeloni, C., & Spencer, J. P. E. (2008). Peroxynitrite induced formation of the neurotoxins 5-*S*-cysteinyl-dopamine and DHBT-1: implications for Parkinson's disease and protection by polyphenols. *Archives of Biochemistry and Biophysics*, *476*, 145–151.
- Vauzour, D., Buonfiglio, M., Corona, G., Chirafisi, J., Vafeiadou, K., Angeloni, C., Hrelia, S., Hrelia, P., & Spencer, J. P. E. (2010). Sulforaphane protects cortical neurons against 5-*S*-cysteinyl-dopamine-induced toxicity through the activation of ERK1/2, Nrf-2 and the upregulation of detoxification enzymes. *Molecular Nutrition & Food Research*, *54*, 532–542.
- Veiga-da-Cunha, M., Tyteca, D., Stroobant, V., Courtoy, P. J., Opperdoes, F. R., & Van Schaftingen, E. (2010). Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). *Journal of Biological Chemistry*, *285*, 18888–18898.
- Verma, V. V., Gupta, R., & Goel, M. (2015). Phylogenetic and evolutionary analysis of functional divergence among Gamma glutamyl transpeptidase (GGT) subfamilies. *Biology Direct*, *10*, 49.
- Visvikis, A., Pawlak, A., Accaoui, M. J., Ichino, K., Leh, H., Guellaen, G., & Wellman, M. (2001). Structure of the 5' sequences of the human γ -glutamyltransferase gene. *European Journal of Biochemistry*, *268*, 317–325.
- Vlaanderen, J., Straif, K., Pukkala, E., Kauppinen, T., Kyyrönen, P., Martinsen, J. I., Kjaerheim, K., Tryggvadottir, L., Hansen, J., Sparén, P., & Weiderpass, E. (2013). Occupational exposure to trichloroethylene and perchloroethylene and the risk of lymphoma, liver, and kidney cancer in four Nordic countries. *Occupational and Environmental Medicine*, *70*, 393–401.
- Vore, M. (1993). Canalicular transport: discovery of ATP-dependent mechanisms. *Toxicology and Applied Pharmacology*, *118*, 2–7.

- Wada, K., Hiratake, J., Irie, M., Okada, T., Yamada, C., Kumagai, H., Suzuki, H., & Fukuyama, K. (2008). Crystal structures of *Escherichia coli* gamma-glutamyltranspeptidase in complex with azaserine and acivicin: novel mechanistic implication for inhibition by glutamine antagonists. *Journal of Molecular Biology*, *380*, 361–372.
- Wada, K., Irie, M., Suzuki, H., & Fukuyama, K. (2010). Crystal structure of the halotolerant gamma-glutamyltranspeptidase from *Bacillus subtilis* in complex with glutamate reveals a unique architecture of the solvent-exposed catalytic pocket. *FEBS Journal*, *277*, 1000–1009.
- Wakabayashi, H., Wakabayashi, M., Eisenreich, W., & Engel, K. H. (2004). Stereochemical course of the generation of 3-mercaptohexanal and 3-mercaptohexanol by β -lyase-catalyzed cleavage of cysteine conjugates. *Journal of Agricultural and Food Chemistry*, *52*, 110–116.
- Wallin, A., Gerdes, R. G., Morgenstern, R., Jones, T. W., & Ormstad, K. (1988). Features of microsomal and cytosolic glutathione conjugation of hexachlorobutadiene in rat liver. *Chemico-Biological Interactions*, *68*, 1–11.
- Wan, L., & O'Brien, P. (2014). Molecular mechanism of 17 α -ethinylestradiol cytotoxicity in isolated rat hepatocytes. *Canadian Journal of Physiology and Pharmacology*, *92*, 21–26.
- Warrander, A., Allen, J. M., & Andrews, R. S. (1985). Incorporation of radiolabelled amino acids into the sulphur-containing metabolites of paracetamol by the hamster. *Xenobiotica*, *15*, 891–897.
- Werner, M., Birner, G., & Dekant, W. (1995a). The role of cytochrome P450A1/2 in the sex-specific sulfoxidation of the hexachlorobutadiene metabolite, *N*-acetyl-*S*-(pentachlorobutadienyl)-L-cysteine in rats. *Drug Metabolism and Disposition*, *23*, 861–868.
- Werner, M., Guo, Z., Birner, G., Dekant, W., & Guengerich, F. P. (1995b). The sulfoxidation of the hexachlorobutadiene metabolite, *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine is catalyzed by human cytochrome P450 3A enzymes. *Chemical Research in Toxicology*, *8*, 917–923.
- Werner, M., Birner, G., & Dekant, W. (1996). Sulfoxidation of mercapturic acids derived from tri- and tetrachloroethene by cytochromes P450 3A: a bioactivation reaction in addition to deacetylation and cysteine conjugate β -lyase mediated cleavage. *Chemical Research in Toxicology*, *9*, 41–49.
- Wessels, H. P., Hansen, G. H., Fuhrer, C., Look, A. T., Sjöström, H., Norén, O., & Spiess, M. (1990). Aminopeptidase M is directly sorted to the apical domain in MDCK cells. *Journal of Cell Biology*, *111*, 2923–2930.
- West, M. B., & Hanigan, M. H. (2010). γ -Glutamyl transpeptidase is a heavily N-glycosylated heterodimer in HepG2 cells. *Archives of Biochemistry and Biophysics*, *504*, 177–181.
- West, M. B., Segu, Z. M., Feasley, C. L., Kang, P., Klouckova, I., Li, C., Novotny, M. V., West, C. M., Mechref, Y., & Hanigan, M. H. (2010). Analysis of site-specific glycosylation of renal and hepatic γ -glutamyl transpeptidase from normal human tissue. *Journal of Biological Chemistry*, *285*, 29511–29524.
- West, M. B., Wickham, S., Quinalty, L. M., Pavlovicz, R. E., Li, C., & Hanigan, M. H. (2011). Autocatalytic cleavage of human γ -glutamyl transpeptidase is highly dependent on N-glycosylation at Asparagine 95. *Journal of Biological Chemistry*, *286*, 28876–28888.
- West, M. B., Wickham, S., Parks, E. E., Sherry, D. M., & Hanigan, M. H. (2013a). Human GGT2 does not autocleave into a functional enzyme: a cautionary tale for interpretation of microarray data on redox signaling. *Antioxidants and Redox Signaling*, *19*, 1877–1888.
- West, M. B., Chen, Y., Wickham, S., Heroux, A., Cahill, K., Hanigan, M. H., & Mooers, B. H. M. (2013b). Novel insights into eukaryotic γ -glutamyl transpeptidase 1 from the crystal structure of the glutamate-bound human enzyme. *Journal of Biological Chemistry*, *288*, 31902–31913.
- Whitfield, J. B. (2001). γ -Glutamyl transferase. *Critical Reviews in Clinical Laboratory Sciences*, *38*, 263–355.
- Wickham, S., West, M. B., Cook, P. F., & Hanigan, M. H. (2011). γ -Glutamyl compounds: substrate specificity of γ -glutamyl transpeptidase enzymes. *Analytical Biochemistry*, *414*, 208–214.
- Wickham, S., Regan, N., West, M. B., Kumar, V. P., Thai, J., Li, P. K., Cook, P. F., & Hanigan, M. H. (2012). Divergent effects of compounds on the hydrolysis and transpeptidation reactions of gamma-glutamyl transpeptidase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *27*, 476–489.
- Wickham, S., Regan, N., West, M. B., Thai, J., Cook, P. F., Terzyan, S. S., Li, P. K., & Hanigan, M. H. (2013). Inhibition of human γ -glutamyl transpeptidase: development of more potent, physiologically relevant, uncompetitive inhibitors. *Biochemical Journal*, *450*, 547–557.
- Williams, R. T. (1959). *Detoxification Mechanisms: The Metabolism and Detoxification of Drugs, Toxic Substances and Other Organic Compounds*. New York, NY: Wiley.
- Wright, S. H., Wunz, T. M., North, J., & Stevens, J. L. (1998). Na-dependent transport of *S*-(1,2-dichlorovinyl)-L-cysteine by renal brush border membrane vesicles. *Journal of Pharmacology and Experimental Therapeutics*, *285*, 162–169.
- Wu, F., & Minteer, S. (2015). Krebs cycle metabolon: structural evidence of substrate channeling revealed by cross-linking and mass spectrometry. *Angewandte Chemie International Edition in English*, *54*, 1851–1854.
- Wu, C., & Schaum, J. (2000). Exposure assessment of trichloroethylene. *Environmental Health Perspectives*, *108*(Suppl. 2), 359–363.
- Wyllie, S., & Fairlamb, A. H. (2011). Methylglyoxal metabolism in trypanosomes and leishmania. *Seminars in Cell and Developmental Biology*, *22*, 271–277.
- Yamamoto, S., Watanabe, B., Hiratake, J., Tanaka, R., Ohkita, M., & Matsumura, Y. (2011). Preventive effect of GGsTop, a novel and selective gamma-glutamyl transpeptidase inhibitor, on ischemia/reperfusion-induced renal injury in rats. *Journal of Pharmacology and Experimental Therapeutics*, *339*, 945–951.
- Yamashita, K., Hitoi, A., Matsuda, Y., Tsuji, A., Katunuma, N., & Kobata, A. (1983a). Structural studies of the carbohydrate moieties of rat kidney γ -glutamyltranspeptidase. An extremely heterogeneous pattern enriched with nonreducing terminal N-acetylglucosamine residues. *Journal of Biological Chemistry*, *258*, 1098–1107.
- Yamashita, K., Hitoi, A., Matsuda, Y., Tsuji, A., Katunuma, N., & Kobata, A. (1983b). Structural studies of the carbohydrate moieties of rat kidney γ -glutamyltranspeptidase. *Journal of Biological Chemistry*, *258*, 1098–1107.
- Yamashita, K., Hitoi, A., Matsuda, Y., Miura, T., Katunuma, N., & Kobata, A. (1986). Structures of sugar chains of human kidney γ -glutamyltranspeptidase. *Journal of Biochemistry*, *99*, 55–62.
- Yaqoob, N., Evans, A., Foster, J. R., & Lock, E. A. (2014). Trichloroethylene and trichloroethanol-induced formic aciduria and renal injury in male F-344 rats following 12 weeks exposure. *Toxicology*, *323*, 70–77.
- Younis, I. R., Elliott, M., Peer, C. J., Cooper, A. J. L., Pinto, J. T., Konat, G. W., Kraszpuski, M., Petros, W. P., & Callery, P. S. (2008). Dehydroalanine analog of glutathione: an electrophilic busulfan metabolite that binds to human glutathione *S*-transferase A1-1. *Journal of Pharmacology and Experimental Therapeutics*, *327*, 770–776.
- Zhang, L., & Hanigan, M. H. (2003). Role of cysteine *S*-conjugate β -lyase in the metabolism of cisplatin. *Journal of Pharmacology and Experimental Therapeutics*, *306*, 988–994.
- Zhang, G. H., & Stevens, J. L. (1989). Transport and activation of *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine in rat kidney proximal tubules. *Toxicology and Applied Pharmacology*, *100*, 51–61.
- Zhang, L., Cooper, A. J. L., Krasnikov, B. F., Xu, H., Bubber, P., Pinto, J. T., Gibson, G. E., & Hanigan, M. H. (2006). Cisplatin-induced toxicity is associated with platinum deposition in mouse kidney mitochondria in vivo and with selective inactivation of α -ketoglutarate dehydrogenase complex in LLC-PK₁ cells. *Biochemistry*, *45*, 8959–8971.
- Ziegler, D. M. (1988). Flavin-containing monooxygenases: catalytic mechanism and substrate specificities. *Drug Metabolism Reviews*, *19*, 1–32.

Relevant Websites

<http://www.atsdr.cdc.gov>—Agency for Toxic Substances and Disease Registry.

<http://nutrigene.4t.com>—Nutrition, Metabolism and Genome Function.