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Endogenous Glutathione Conjugates: Occurrence and Biological Functions

WEI WANG AND NAZZARENO BALLATORI[a]

Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, New York

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I. Introduction

Glutathione (GSH)[b] performs a variety of important physiological and metabolic functions in all mammalian cells, including the detoxification of free radicals, metals, and other electrophilic compounds. One important detoxification mechanism involves the binding of GSH to electrophilic chemicals and the export of the resulting GSH S-conjugates from the cell. These conjugation reactions have been extensively characterized for a multitude of foreign chemicals, but they are also critical for the metabolism of endogenous reactive intermediates and for the formation of specific biological mediators. GSH forms thioether conjugates with leukotrienes, pros-

[a] Address for correspondence: Ned Ballatori, Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642. E-mail: Ballatorin@envmed.rochester.edu.

[b] Abbreviations: α-MeDA, α-methyldopamine; γGT, γ-glutamyl transpeptidase; ATP, adenosine triphosphate; CoASSG, GSH-coenzyme A mixed disulfide; DCE-SG, S-(12-Dicarboxyethyl)glutathione; DNA, deoxyribonucleic acid; EDRF, endothelium-derived relaxing factor; GS-NO, S-nitrosoglutathione; GSH, reduced glutathione; GspdSH, N^1-monoglutathionylspermidine; GSSG, glutathione disulfide; HHE, 4-hydroxyhexenal; HNE, 4-hydroxynonenal; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; MDA, 34-(±)-(methylenedioxy)amphetamine; MeDA-SG, 5-(S-glutathionyl)-α-methyldopamine; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PGA, prostaglandin A; PGD, prostaglandin D; PGE, prostaglandin E; PGH, prostaglandin H.

taglandins, hepoxilin, nitric oxide, hydroxyalkenals, ascorbic acid, dopa, dopamine, and maleic acid, and it forms thioesters with cysteine, coenzyme A, proteins, and other cellular thiols. The glycine carboxyl group of GSH binds to the amino group of spermidine to produce GSH-amides in reactions catalyzed by glutathionylspermidine synthetase and trypanothione synthetase in bacterial systems. GSH also binds endogenous metals, such as copper, selenium, chromium, and zinc, via nonenzymatic reactions.

The binding of GSH to these endogenous compounds serves several important roles: (*a*) it serves to limit and regulate the reactivity of the chemicals; (*b*) it facilitates their membrane transport and elimination from the cell and organism; and (*c*) in some cases, it leads to the formation of essential biological mediators. The cysteinyl leukotrienes, for example, are involved in inflammatory and anaphylactic reactions. The GSH conjugate of 9-deoxy-Δ^9, Δ^{12}-prostaglandin D_2 may modulate the antiproliferative activity of the parent compound (Atsmon *et al.*, 1990a), whereas S-nitrosoglutathione, a relatively stable intermediate derived from the nonenzymatic reaction of nitric oxide with intracellular GSH, seems to have the same biological functions as nitric oxide itself (Ignarro, 1990).

Many proteins are activated or inhibited in vitro by the disulfide exchange between the protein and GSH. Glutathione disulfide (GSSG) can activate enzymes such as glucose-6-phosphatase, acid phosphatase, γ-aminolaevulinate synthetase, and fructose 16-bisphosphatase; however, it inhibits glycogen synthetase, pyruvate kinase, adenylate cyclase, phosphorylase/phosphatase, ribonucleotide reductase, phosphofructokinase, glycogen debranching enzyme, and fatty acid synthase.

The present discussion reviews the occurrence and biological functions of the endogenous GSH S-conjugates.

II. Glutathione Homeostasis

GSH is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) that serves several essential functions within the cell (Ballatori, 1994; Deleve and Kaplowitz, 1991; Meister and Tate, 1976; Meister and Anderson, 1983). It is the most abundant nonprotein thiol in almost all aerobic species, occurring at intracellular concentrations of 0.5 to 10 mM. In contrast, extracellular GSH concentrations are usually 3 to 4 orders of magnitude lower. Under physiological conditions, GSSG reductase maintains more than 98% of intracellular GSH in the reduced, thiol form (GSH). The rest is present within the cell as mixed disulfides (mainly GS-S-protein), as the disulfide (GSSG), and as thioethers.

The key functional element of the GSH molecule is the cysteinyl moiety, which provides the reactive thiol group and is responsible for the many functions of GSH. These functions include (*a*) the maintenance of protein structure and function by reducing the disulfide linkages of proteins, (*b*) the regulation of protein synthesis and degradation, (*c*) the maintenance of immune function, (*d*) protection against oxidative damage, and (*e*) detoxification of reactive chemicals. GSH also serves as a storage and transport form of the cysteine moiety, and it functions in (*a*) leukotriene and prostaglandin metabolism, (*b*) the reduction of ribonucleotides to deoxyribonucleotides, (*c*) the modulation of microtubule-related processes, and (*d*) bile formation (for reviews see Deleve and Kaplowitz, 1991; Gilbert, 1982; Hinchman and Ballatori, 1994; Kosower and Kosower, 1978; Meister and Tate, 1976; Meister and Anderson, 1983).

The key structural elements of GSH are the γ-carboxyl peptide linkage of glutamate and the presence of the C-terminal glycine, which directly determine its metabolism and function. The N-terminal glutamyl and cysteinyl moieties are linked through the γ-carboxyl group of glutamate instead of the more common α-carboxyl peptide linkage, restricting cleavage to γ-glutamyl transpeptidase (γGT), which occurs on the external surface of certain cell membranes. Therefore, GSH is resistant to intracellular degradation and can only be cleaved by cell types that have γGt on the cell membrane. The presence of the C-terminal glycine protects the peptide against cleavage by intracellular γ-glutamylcyclotransferase.

A. Synthesis and Degradation

GSH is synthesized in all mammalian cells (Meister and Tate, 1976), and the liver is a major site of biosynthesis (Deleve and Kaplowitz, 1991). The synthesis of GSH from its three amino acid precursors L-glutamate, L-cysteine, and glycine takes place in the cytosol (fig. 1). It is a two-step reaction, catalyzed by γ-glutamylcysteine synthetase and by GSH synthetase, that requires

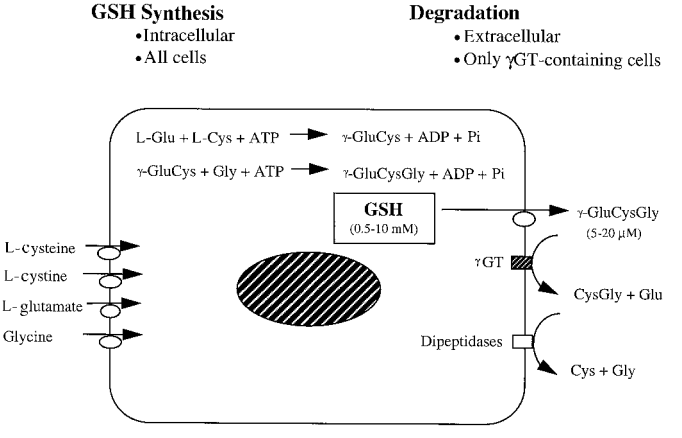

FIG. 1. GSH synthesis and degradation. In the first step of GSH synthesis, an amide linkage is formed between cysteine and glutamate catalyzed by the γ-glutamylcysteine synthetase. GSH synthetase then catalyzes the reaction between glycine and the cysteine carboxyl of γ-glutamylcysteine dipeptide to form GSH. GSH is transported out of the cell and broken down by the membrane-bound enzyme γGT, which removes the γ-glutamyl moiety, and by dipeptidases, which remove the glycine moiety. The resulting amino acids can be reabsorbed and used for additional GSH synthesis. ADP, adenosine 5′diphosphate; Cys, cysteine; Glu, glutamate; Gly, glycine; Pi, inorganic phosphate.

two moles of adenosine triphosphate (ATP) per mole of GSH (fig. 1). The first step, catalyzed by γ-glutamylcysteine synthetase, is controlled by negative feedback from its end product, GSH (Richman and Meister, 1975). However, feedback inhibition can be partially prevented by an excess of glutamate that blocks the regulatory site on the enzyme (Meister, 1984; Meister and Anderson, 1983; Richman and Meister, 1975). When GSH is consumed and feedback inhibition is lost, the availability of cysteine as a precursor can become the limiting factor.

Although GSH is synthesized inside the cell, its biodegradation occurs outside of cells (fig. 1). The enzymes that catalyze its breakdown are γGT and dipeptidases, which are membrane-bound proteins that are located predominantly on the apical surface of epithelial tissues. γGT is the only enzyme that removes the γ-glutamyl moiety from GSH under physiological conditions. Dipeptidases remove the glycyl moiety. The breakdown products (glutamate, glycine, and cysteine) can be reabsorbed into the cell for GSH synthesis (fig. 1).

The GSH S-conjugates are metabolized by the same degradative enzymes that metabolize GSH (fig. 2). The breakdown products of the GSH S-conjugates are glutamate, glycine, and cysteine, which can also be reabsorbed into the cell. The glutamate and glycine may then be used for GSH synthesis, whereas the cysteine S-conjugates can be acetylated on the amino group of the cysteinyl residue by intracellular N-acetyltransferases to form the corresponding mercapturic acids (N-acetylcysteine S-conjugates). Mercapturic acids are released into the circulation or bile (Hinchman *et al.*, 1991); some are eventually excreted in urine, and some may undergo further metabolism. The addition of the N-acetylcysteine moiety generally increases a compound's polarity and water solubility, and converts neutral compounds to anions, facilitating their transport across cell membranes and their excretion from the organism (Boyland and Chasseaud, 1969).

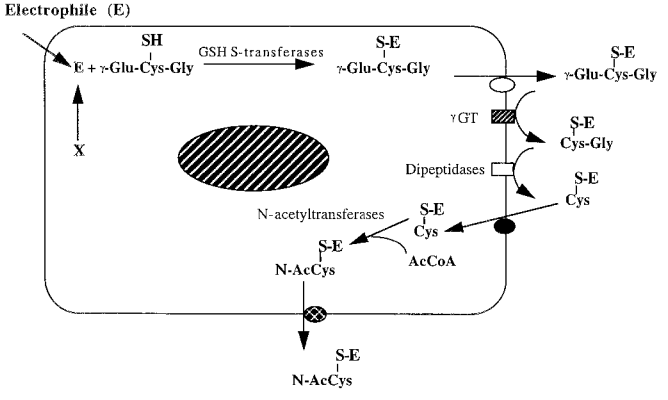

FIG. 2. Metabolic and transport steps in mercapturic acid biosynthesis. The GSH S-conjugate is made intracellularly and then transported out of the cell for subsequent degradation by the ectoproteins like γGT and the dipeptidases. The cysteine S-conjugate that is formed is transported back into the cell and N-acetylated to form mercapturic acid. (This figure was adapted with permission from Hinchman and Ballatori, 1994, *J Toxicol Environ Health* **41:**387–409.) Ac, acetyl; CoA, coenzyme A; Cys, cysteine; E, electrophile; Glu, glutamate; Gly, glycine; S-E, thiol-conjugated electrophile; SH, sulfhydryl group.

B. Biological Functions

1. Regulation of cellular sulfhydryl status.

a. REDOX REACTIONS. All aerobic organisms are subject to physiological oxidant stress as a consequence of aerobic metabolism. The intermediates that are formed, including superoxide and hydrogen peroxide, lead to the further production of toxic oxygen radicals that can cause lipid peroxidation and disrupt metabolic processes. GSH is the predominant defense against these toxic products of oxygen, particularly in the mitochondria, a major site for the synthesis of reactive oxygen intermediates (Deleve and Kaplowitz, 1991). Mitochondrial GSH is critical in the defense against both physiologically and pathologically generated oxidant stress. Mitochondria do not have the enzymes necessary for GSH synthesis, and they import cytosolic GSH. Because catalase is compartmentalized in the peroxisome, mitochondrial GSH in the presence of GSH peroxidase is the main defense against toxic oxygen intermediates.

Endogenously produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase (fig. 3). As a consequence, GSH is oxidized to GSSG, which in turn is rapidly reduced back to GSH by GSSG reductase at the expense of reduced nicotinamide adenine dinucleotide phosphate (NADPH), thereby forming a closed system (redox cycle) as illustrated in figure 3. The reduction of organic hydroperoxides by GSH may be catalyzed by either this selenium-dependent GSH peroxidase or by selenium-independent peroxidase activity, which is provided by GSH S-transferases of the α class. To maintain the redox state of the cell, the GSSG generated during oxidant stress must be reduced by GSSG reductase, using reducing equivalents from NADPH.

Under normal conditions, GSSG reductase is quite effective at maintaining most cellular GSH in its reduced state (more than 98% GSH). However, under severe oxidant stress or where GSSG reductase activity is impaired, the ability of the cell to reduce GSSG may be overwhelmed, leading to its accumulation within the cytosol. To protect itself from a shift in redox equilibrium, the cell can actively transport GSSG out of the cell. However, GSSG may also react with cellular protein sulfhydryls via a mixed disulfide reaction, a process that can result in impaired protein function.

b. THIOL-DISULFIDE EXCHANGE. As oxygen tension in the environment increased during evolution, aerobic organisms needed a system to restore key sulfhydryl groups to their reduced state after exposure to oxidant stress (Fridovich, 1989; Naqui and Chance, 1986; Sohal and Weindruch, 1996; Sundquist and Fahey, 1989). Without a process to reduce protein disulfides, vulnerable cysteinyl residues of essential enzymes might remain oxidized, leading to changes in catalytic activity.

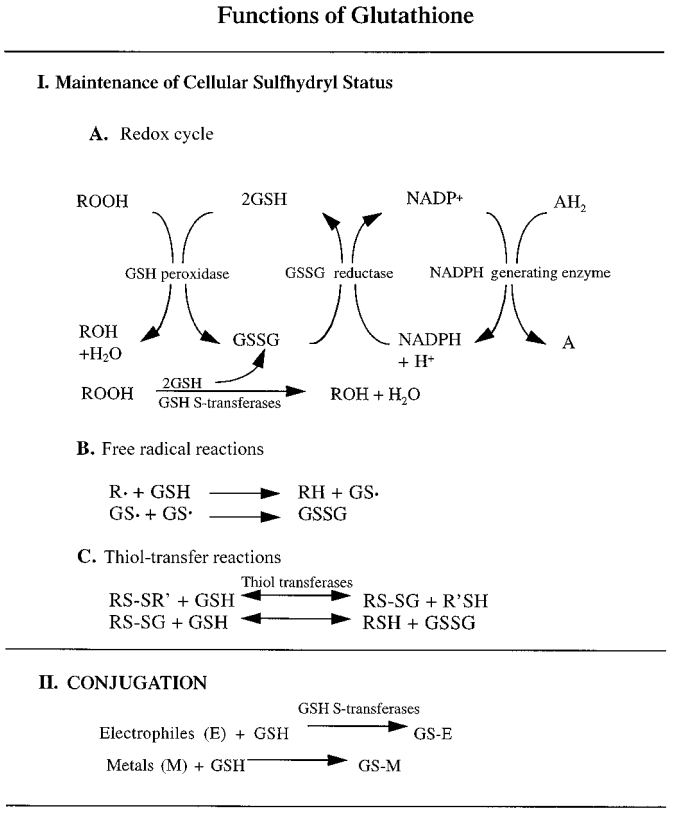

FIG. 3. The detoxification functions of GSH include the conjugation of electrophilic chemicals and reactive metals and the maintenance of the cellular thiol redox status. AH_2 and A, reduced and oxidized forms, respectively, of compounds that participate in the synthesis of NADPH; GSH, reduced glutathione; GSSG, glutathione disulfide; NADPH and $NADP^+$, reduced and oxidized forms, respectively, of nicotinamide adenine dinucleotidephosphate; RH and R, reduced and oxidized forms, respectively, of some organic molecules; ROOH, a hydroperoxide; RDH, an alcohol; RS-SG, a glutathione thioester; RS-SR′, a mixed disulfide of two organic molecules.

This function is fulfilled by the thiol-disulfide exchange catalyzed by thiol-transferases in the presence of GSH, and may be essential to aerobic life (fig. 3).

The thiol-disulfide equilibrium within the cell may regulate certain metabolic pathways by activating or inactivating key enzymes. Whereas many proteins are active when the key sulfhydryls are in the thiol form, others require them to be in the oxidized, disulfide form. Because the thiol-transferase reaction is bidirectional, the equilibrium will be determined by the redox state of the cell.

c. STORAGE AND TRANSFER OF CYSTEINE. Cysteine autooxidizes rapidly to cystine, producing potentially toxic oxygen radicals (Olney *et al.*, 1990). To avoid the toxicity of the autooxidation reaction, most of the nonprotein cysteine is stored as GSH. The liver and kidney play a major role in the homeostasis of GSH and cysteine (Deleve and Kaplowitz, 1991).

2. Conjugation of electrophiles and metals. GSH plays a major role in detoxifying many reactive metabolites by either spontaneous conjugation or by a reaction catalyzed by the GSH S-transferases (Boyland and Chasseaud, 1969; Coles and Ketterer, 1990; Hinchman and Ballatori, 1994; Jakoby, 1978; Mannervik, 1985). GSH S-transferases have broad and overlapping substrate specificities, which allow them to participate in the detoxification of a chemically diverse group of compounds. The most common reactions involve nucleophilic attack by GSH on an electrophilic carbon: saturated carbon atoms (e.g., alkyl halides, lactones and epoxides), unsaturated carbon atoms (e.g., α, β-unsaturated compounds, quinones and quinonimines, and esters), or aromatic carbon atoms (e.g., aryl halides and aryl nitro compounds; Douglas, 1988). The substrates have in common a degree of hydrophobicity and possess electrophilic centers that undergo nucleophilic substitution, nucleophilic addition to α, β-unsaturated ketones or epoxides or, in the case of hydroperoxides, nucleophilic attack on electrophilic oxygen, resulting in reduction.

GSH S-transferases are a family of multifunctional enzymes present in the cytosol of most cells as homodimeric or heterodimeric proteins, with subunit molecular weights ranging from 24,000 to 27,500 daltons (Da) (Armstrong, 1987; Mannervik, 1985; Ketterer *et al.*, 1985; Hayes and Mantle, 1986). Using structural properties, immunoreactivity, and substrate specificities, three different classes of mammalian cytosolic GSH S-transferases have been identified and named: α, μ, and π (Mannervik, 1985). More recently, a fourth class, τ, was introduced by Meyer *et al.* (1991). GSH S-transferase enzymes have two active sites per dimer that behave independently of one another. Each active site consists of at least two ligand binding regions. The GSH binding site is very specific for this tripeptide, whereas the binding site for the electrophilic substrate is less specific (Danielson and Mannervik, 1985).

GSH also forms metal complexes via nonenzymatic reactions (Ballatori, 1994). GSH is one of the most versatile and pervasive metal binding ligands and plays an important role in metal transport, storage, and metabolism. GSH works (*a*) in the mobilization and delivery of metals between ligands, (*b*) in the transport of metal across cell membranes, (*c*) as a source of cysteine for metal binding, and (*d*) as a reductant or cofactor in redox reactions involving metals. The sulfhydryl group of the cysteine moiety of GSH has a high affinity for metals, forming thermodynamically stable but kinetically labile mercaptides with several metals, including mercury, silver, cadmium, arsenic, lead, gold, zinc, and copper.

Conjugation with GSH is not always protective but may actually activate compounds. For example, the GSH conjugation of dibromoethane, which is used as a lead scavenger in leaded gas, forms a 2-bromo-thioether, which is subsequently transformed into a highly reactive, mutagenic, and carcinogenic intermediate, possibly an episulfonium (Rannug, 1980; Van Bladeren *et al.*, 1981; Rannug *et al.*, 1978). Sulfur mustards are capable of alkylating nucleophilic sites in proteins and deoxyribonucleic acid (DNA). Other classes of compounds, in-

cluding nephrotoxic haloalkenes, quinones, and isothiocyanates, are also converted by GSH conjugate formation to toxic metabolites (Anders *et al.*, 1987; Vroomen *et al.*, 1988; Horvath *et al.*, 1990; Koob and Dekant, 1991).

III. Endogenous Glutathione Thioethers

In addition to the many exogenous electrophilic chemicals, a multitude of endogenous compounds also react with GSH to form adducts (tables 1 and 2). Some of the endogenous electrophiles are produced as byproducts of lipid peroxidation, which may be caused by free radicals or high-energy irradiation (Slater, 1984). Many of these compounds are cytotoxic or genotoxic. Without detoxification, the result is a cascade of reactions leading to further radical damage, membrane decomposition, and free-radical attack on other cellular targets, such as DNA.

A. Leukotriene C_4 (LTC_4)

1. Synthesis and biological actions. Leukotrienes are potent biological mediators that are formed in response to a variety of immunological and inflammatory stimuli (Denzlinger *et al.*, 1985; Hammarstrom, 1983; Lewis and Austen, 1984; Piper, 1984; Samuelsson, 1983). They are synthesized by a variety of white blood cells, including macrophages, monocytes, neutrophils, eosinophils, and mast cells (connective tissue cells derived from the blood-forming tissues that secrete substances that mediate inflammatory and allergic reactions), as well as cells in the lung, spleen, brain, and heart (Samuelsson, 1983; Lewis and Austen, 1984; Hammarstrom, 1983; Piper, 1984). In addition, Kupffer cells release $LTC_4(D_4)$-like material upon stimulation (Decker, 1985). After production, leukotrienes are released by the cells and can be detected in various body fluids.

As illustrated in figure 4, leukotrienes are derived from arachidonic acid, a polyunsaturated fatty acid that is abundant in biological membranes. First, arachidonic acid is released from the sn-2 position of membrane phospholipids upon cell stimulation. After oxygenation and subsequent dehydration catalyzed by 5-lipoxygenase and 5-lipoxygenase-activating protein (FLAP), arachidonic acid is converted to the unstable epoxide leuko-

TABLE 1
Endogenous glutathione thioethers

Glutathione-thioethers	Parent compound	Tissue	Reference
Leukotriene C_4	Leukotriene A_4	Multiple	
S-PGJ_2	PGJ_2	Multiple	Atsmon *et al.* (1990b)
S-(Δ^{12}-PGJ_2)	Δ^{12}-PGJ_2	CHO cells	Atsmon *et al.* (1990b)
		Rat hepatocytes	Atsmon *et al.* (1990b)
		Porcine aortic endothelial cells	Koizumi *et al.* (1992)
S-(9-deoxy-Δ^{12}-PGD_2)	9-deoxy-Δ^{12}-PGD_2		Atsmon *et al.* (1990b)
S-PGA_2	PGA_2	Mouse leukemia cells	Parker and Ankel (1992)
S-PGA_1	PGA_1	Human and rat liver	Cagen *et al.* (1975)
		Human red blood cells	Cagen *et al.* (1976)
S-hepoxilin A_3	Hepoxilin A_3	Rat brain	Pace-Asciak *et al.* (1990)
S-(4-hydroxynonenal)	4-hydroxynonenal	Ehrlich mouse ascites cells	Grune *et al.* (1994)
S-(4-hydroxyhexenal)	4-hydroxyhexenal		Winter *et al.* (1987)
S-palmityl	Palmityl CoA Palmityl adenylate	Rat brain and liver	Vignais and Zabin (1958)
13-oxooctadecadienoic acid	Linoleic acid		Blackburn *et al.* (1997)
S-menadione	Vitamin K_3	Bovine pulmonary artery endothelial cells	Chang *et al.* (1992)
S-(17-β-estradiol)	17-β-estradiol	Hamster hepatic microsomes	Butterworth *et al.* (1996) Butterworth *et al.* (1998)
S-cholesterol-5,6-oxide	Cholesterol-5,6-oxide	Rat liver	Meyer and Ketterer (1982)
S-nitroso	Nitric oxide	Human neutrophils and airways	Gaston *et al.* (1993)
S-dopa	Dopa	Malignant melanoma	Agrup *et al.* (1977)
S-dopamine	Dopamine	Brain	Fornstedt *et al.* (1986)
S-α-methyldopamine	α-methyldopamine	Brain	Miller *et al.* (1995)
S-β-alanyl-dopa	β-alanyl-dopa	Adult Sarcophaga (flesh fly)	Leem *et al.* (1996)
S-methyl	Unknown	Bovine brain	Kanazawa *et al.* (1965)
S-ethyl	Unknown		Fedtke *et al.* (1994)
S-hydroxyethyl	Unknown		Zhang *et al.* (1995)
S-(1,2-dicarboxyethyl)	Maleic acid	Calf lens	Calam and Waley (1963)
		Rat liver	Tsuboi *et al.* (1990a)
		Rat heart	Tsuboi *et al.* (1990a)
S-(N-hydroxy-N-methylcarbamoyl)	Methylglyoxal		Hamilton and Creighton (1992)
S-acetyl	Acetyl CoA		Stern and Drummond (1961)
S-acetoacetyl	Acetoacetyl CoA		Stern and Drummond (1961)
7-S-glutathionyl-tryptamine-4,5-dione	5-hydroxytryptamine	Brain	Wong *et al.* (1993)
4-S-glutathionyl-5-hydroxytryptophan	5-hydroxytryptophan	Cerebrospinal fluid	Wu and Dryhurst (1996)
7-S-glutathionyl-tryptophan-4,5-dione	5-hydroxytryptophan	Cerebrospinal fluid	Wu and Dryhurst (1996)
S-[2-carboxy-1-(1H-imidazol-4-yl)ethyl]	Trans-urocanic acid		Kinuta *et al.* (1993)
S-anthocyanins	Anthocyanins	Plants	Marrs *et al.* (1995)

CHO, chinese hamster ovary; CoA, coenzyme A.

TABLE 2
Endogenous glutathione amides, thioesters, and mercaptides

Glutathione-thioethers	Parent compound	Tissue	Reference
Amides			
N^1-monoglutathionyl spermidine	Spermidine	*Escherichia coli*	Tabor and Tabor (1975)
		Trypanosoma and *leishmania*	Fairlamb *et al.* (1985)
N^1,N^8-bis(glutathionyl) spermidine	Spermidine	*Trypanosoma* and *leishmania*	Fairlamb *et al.* (1985)
Thioesters			
GS-SG	GSH	Multiple	
GS-S-cysteine	Cysteine	Multiple	
GS-S-cysteinylglycine	Cysteinylglycine	Multiple	
GS-S-O_3H	Sulphate	Rat intestine	Robinson and Pasternak (1964)
GS-S-CoA	CoA	Rat liver	Ondarza and Aubanel (1960)
		Bovine liver	Wilken and Hansen (1961)
GS-S-proteins	Proteins	Multiple	
S-carbonic anhydrase III	Carbonic anhydrase III		Cabiscol and Levine (1996)
S-actin	Actin		Drewes and Faulstich (1990)
Mercaptides			
GS-Cu(I)	Cu(I)	Multiple	Freedman *et al.* (1989)
GS-Cu(II)-SG	Cu(II)	Multiple	Freedman *et al.* (1989)
GS-SeH	Se	Multiple	Vernie *et al.* (1979)
GS-Se-SG	Se	Multiple	Vernie *et al.* (1979)
GS-Zn-R	Zn	Multiple	Ciriolo *et al.* (1990)
GS-Cr-R	Cr	Multiple	Standeven and Wetterhahn (1991)

GS, glutathione moiety; CoA, coenzyme A; R, metal ligand; GS-S, glutathione mixed disulfide; GSH, glutathione.

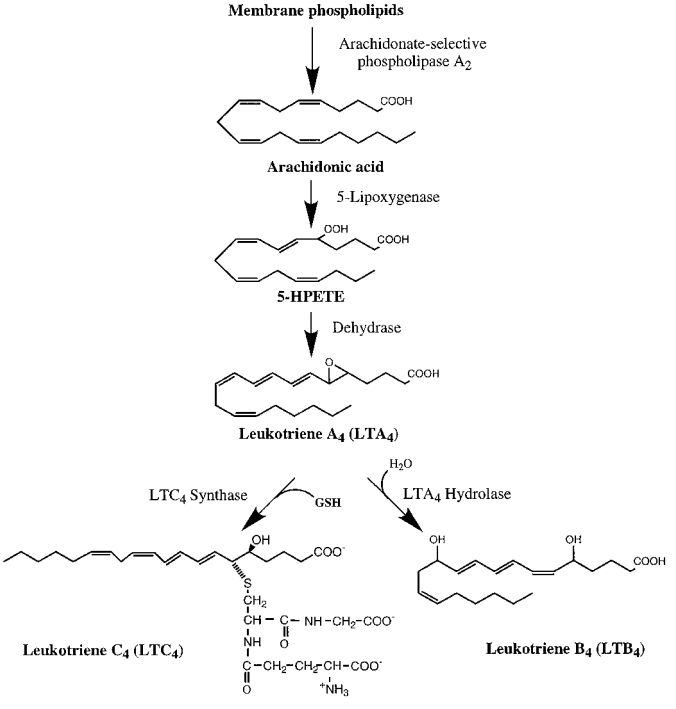

FIG. 4. The formation of LTA_4 from arachidonic acid via the 5-lipoxygenase pathway and the conversion of LTA_4 to LTC_4 and LTB_4. 5-HPETE, 5-hydroperoxyeicosatetraenoic acid.

triene A_4 (LTA_4; Rouzer *et al.*, 1986; Dixon *et al.*, 1990). As illustrated in figure 4, LTA_4 can be metabolized by two routes. The first involves stereoselective hydrolysis to leukotriene B_4 (LTB_4) by the cytosolic enzyme LTA_4 hydrolase. LTB_4 is a potent chemotactic agent involved in attracting certain types of white blood cells to fight infection and is an important mediator of inflammatory reactions (Keppler *et al.*, 1985). The immunoregulatory effects of LTB_4 include inhibiting the proliferation of T-lymphocytes (Payan *et al.*, 1984) and stimulating the activity of natural cytotoxic cells (Rola-Pleszczynski *et al.*, 1983).

The second pathway involves the conjugation of LTA_4 with GSH by the membrane-bound enzyme LTC_4 synthase, to form leukotriene C_4 (LTC_4). LTC_4 synthase is a unique membrane-bound enzyme that catalyzes the committed step in the biosynthesis of all of the peptidoleukotrienes (Nicholson *et al.*, 1993). LTC_4 synthase conjugates GSH with the unstable epoxide LTA_4 to form LTC_4 and, as such, is a GSH S-transferase activity. Like other known GSH S-transferases, LTC_4 synthase is enzymatically active as a multimer composed of low molecular mass subunits (Nicholson *et al.*, 1993). However, unlike other members of the GSH S-transferases multigene family, LTC_4 synthase does not seem to be involved in the metabolism of xenobiotics, but rather seems to be exclusively committed to the biosynthesis of LTC_4 (Nicholson *et al.*, 1992). Welsch *et al.* (1994) recently reported the molecular cloning of LTC_4 synthase from the human monocytic leukemia cell line THP-1 and the expression of the active enzyme in bacterial, insect, and mammalian cells. LTC_4 synthase seems to be a unique GSH S-transferase that shows no similarity to other GSH S-transferases. However, the amino acid sequence of this protein is strikingly similar to 5-lipoxygenase activating protein (FLAP), a protein involved in the metabolism of arachidonic acid (31% identity, 53% similarity).

LTC_4 is itself converted to leukotriene D_4 (LTD_4) by γGT via the removal of glutamic acid (fig. 5). A dipeptidase removes glycine, converting LTD_4 to leukotrienes E_4 (LTE_4). LTE_4 can be N-acetylated to form the mercapturic acid N-acetyl-LTE_4 (fig. 5) or can react with γGT and GSH, resulting in the formation of γ-glutamyl-LTE_4 (LTF_4; Maycock *et al.*, 1989).

The cysteine-containing leukotrienes are involved in inflammatory and anaphylactic reactions (Samuelsson,

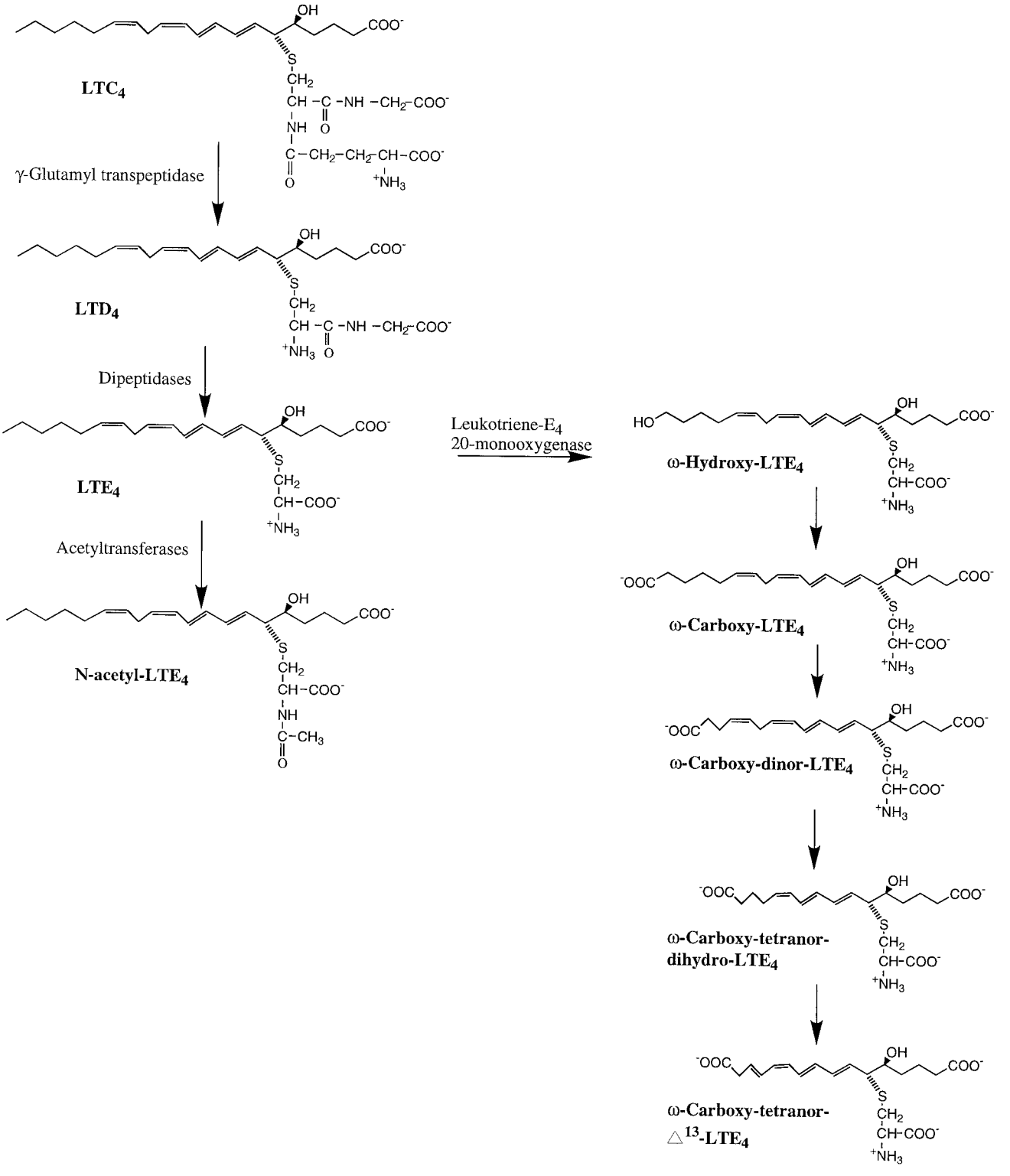

FIG. 5. Structures of LTC_4 and its degradation products. The sequential cleavage of glutamate and glycine from LTC_4 by the ectoenzymes γGT and dipeptidase yield LTD_4 and LTE_4, respectively. The acetylation of LTE_4 yields N-acetyl-LTE_4. Intracellular ω-oxidation of LTE_4 results in the formation of ω-hydroxy-LTE_4 and ω-carboxy-LTE_4. Subsequent β-oxidation from the ω-end leads to ω-carboxy-dinor-LTE_4 and ω-carboxy-tetranor-dihydro-LTE_4.

1983; Lewis and Austen, 1984; Hammarstrom, 1983; Piper, 1984; Feuerstein, 1984). They were isolated and identified as the active mediators of the slow reacting substances of anaphylaxis, which is a violent and potentially fatal allergic reaction; they are released from the lung tissue of asthmatics upon exposure to specific allergens. These substances produce the contraction of vascular, respiratory, and intestinal smooth muscle at

very low concentrations (as little as 10^{-10} M) with a wide range of actions on organ blood flow, airway function, and microvascular permeability. In the respiratory system, leukotrienes constrict bronchi (especially the smaller airways), increase mucus secretion, and are thought to be the mediators in asthma. They are also implicated in immediate hypersensitivity (allergic) reactions, inflammatory reactions, and heart attacks (Piper, 1984; Lewis *et al.*, 1990). Moreover, leukotrienes may be key mediators in inflammatory liver diseases (Keppler *et al.*, 1985). Because of the important role that these mediators may play in the pathophysiology of asthma, compounds that inhibit the production or action of these mediators may be useful for the amelioration of asthma (Cloud *et al.*, 1989).

2. Leukotriene metabolism. The rapid removal of the cysteinyl leukotrienes in inflamed tissues is an important mechanism for their inactivation. The cysteinyl leukotrienes are inactivated in primates by (*a*) intravascular degradation, (*b*) hepatic and renal uptake from the blood circulation, (*c*) intracellular metabolism, and (*d*) biliary and renal excretion of LTC_4 degradation products (Huber *et al.*, 1990)

LTC_4 entering the blood circulation is rapidly metabolized to LTD_4 and LTE_4, and the products are taken up by the liver and kidneys (Denzlinger *et al.* 1986a; Orning *et al.*, 1986). After partial intracellular metabolism, they are excreted into bile and urine. Part of the cysteinyl leukotrienes excreted into bile undergo enterohepatic circulation; the physiological role of this is not fully understood (Denzlinger *et al.*, 1986a). In rodents, intracellular LTE_4 metabolism includes N-acetylation, followed by β-oxidation from the ω-end. Leukotriene metabolites identified in monkey bile or urine after intravenous administration of LTC_4 include LTD_4, LTE_4, ω-hydroxy-LTE_4, ω-carboxy-LTE_4, ω-carboxy-dinor-LTE_4, and ω-carboxy-tetranor-dihydro-LTE_4 (fig. 5).

Huber *et al.* (1990) analyzed the cysteinyl leukotriene metabolites excreted into the bile and urine of monkeys and humans. They found that hepatobiliary leukotriene elimination predominated over renal excretion in both species. In monkeys, large amounts of ω- and β-oxidation products derived from LTE_4 were detected in urine, with ω-carboxy-tetranor-dihydro-LTE_4 being the major LTC_4 metabolite. LTE_4 was the predominant metabolite in bile, and large amounts of ω-oxidation products were also found in bile. The LTC_4 degradation products observed in monkeys were also identified in human bile and urine. The predominant LTC_4 metabolite in human urine was LTE_4. N-acetyl-LTE_4 was a minor metabolite, which presumably was formed by intracellular N-acetylation of LTE_4 in the kidney (Bernstrom and Hammarstrom, 1986). LTD_4 and LTE_4 were the predominant metabolites in human bile. In addition, LTC_4 was not detected in either the bile or the urine of both monkeys and humans. This is most likely a result of the rapid metabolism of LTC_4 to LTD_4 and LTE_4 by ectoenzymes, such as γGT and the dipeptidases (Denzlinger *et al.*, 1986b; Tagari *et al.*, 1989; Hammarstrom *et al.*, 1981). The catabolism of LTC_4 and LTD_4 takes place not only in the blood circulation but also in the bile canaliculi and renal tubules.

B. Prostaglandins

Almost all mammalian cells produce prostaglandins (Eling *et al.*, 1990; Smith, 1992). They were first identified in human semen in the early 1930s via their ability to stimulate uterine contractions and lower blood pressure. The physiological effects of prostaglandins have now been well described; they are similar to those produced by other eicosanoids, such as leukotrienes. They are involved in the inflammatory response, the production of pain and fever, the regulation of blood pressure, the induction of blood clotting, the control of several reproductive functions (such as the induction of labor), and the regulation of the sleep/wake cycle.

Prostaglandins are synthesized from the same precursor as leukotrienes, arachidonic acid, in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase (Miyamoto *et al.*, 1976; fig. 6). This enzyme has two catalytic activities: a cyclooxygenase activity and a hydroperoxidase activity. The former catalyzes the addition of two molecules of oxygen to arachidonic acid, forming prostaglandin G_2. The latter mediates a GSH-dependent reaction that converts the hydroperoxy function of prostaglandin G_2 to a hydroxyl group and forms prostaglandin H_2 (PGH_2). PGH_2 is the immediate precursor of all of the major biologically active products of this pathway, including those that form prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), and prostaglandin $F_{2\alpha}$. (fig. 6). Christ-Hazelhof *et al.* (1976) found that GSH S-transferase isozymes had PGH_2-converting activity; this was further confirmed by Ujihara *et al.* (1988). They demonstrated that PGD_2, PGE_2, and prostaglandin $F_{2\alpha}$ were formed from PGH_2 by various rat GSH S-transferase isozymes.

PGE_2 can be transformed to the dehydration product prostaglandin A_2 (PGA_2), and PGE_1 to PGA_1 (fig. 7; Santoro *et al.*, 1986; Turner *et al.*, 1982; Suzuki *et al.*, 1988; Ikai *et al.*, 1987). Both PGA_1 and PGA_2 are potentially toxic, and GSH can conjugate with both to form S-(PGA)-GSH derivatives (Bogaards *et al.*, 1997; Cagen *et al.*, 1975; Ham *et al.*, 1975; fig. 7). The inhibitory effect of PGA on the proliferation of tumor cells was markedly diminished when GSH was added to cell culture medium containing PGA (Honn and Marnett, 1985). Conversely, GSH-depleted cells are insensitive to the cytotoxicity of PGA_2, suggesting that PGA_2-GSH conjugates may be involved in the cytotoxicity of PGA_2 (Parker and Ankel, 1992). Parker and Ankel (1992) demonstrated that PGA_2 is initially conjugated to GSH and then reduced at the 9-keto position to form S-(9-hydroxy-PGA_2)-GSH. This conjugate is then secreted from the cells and is

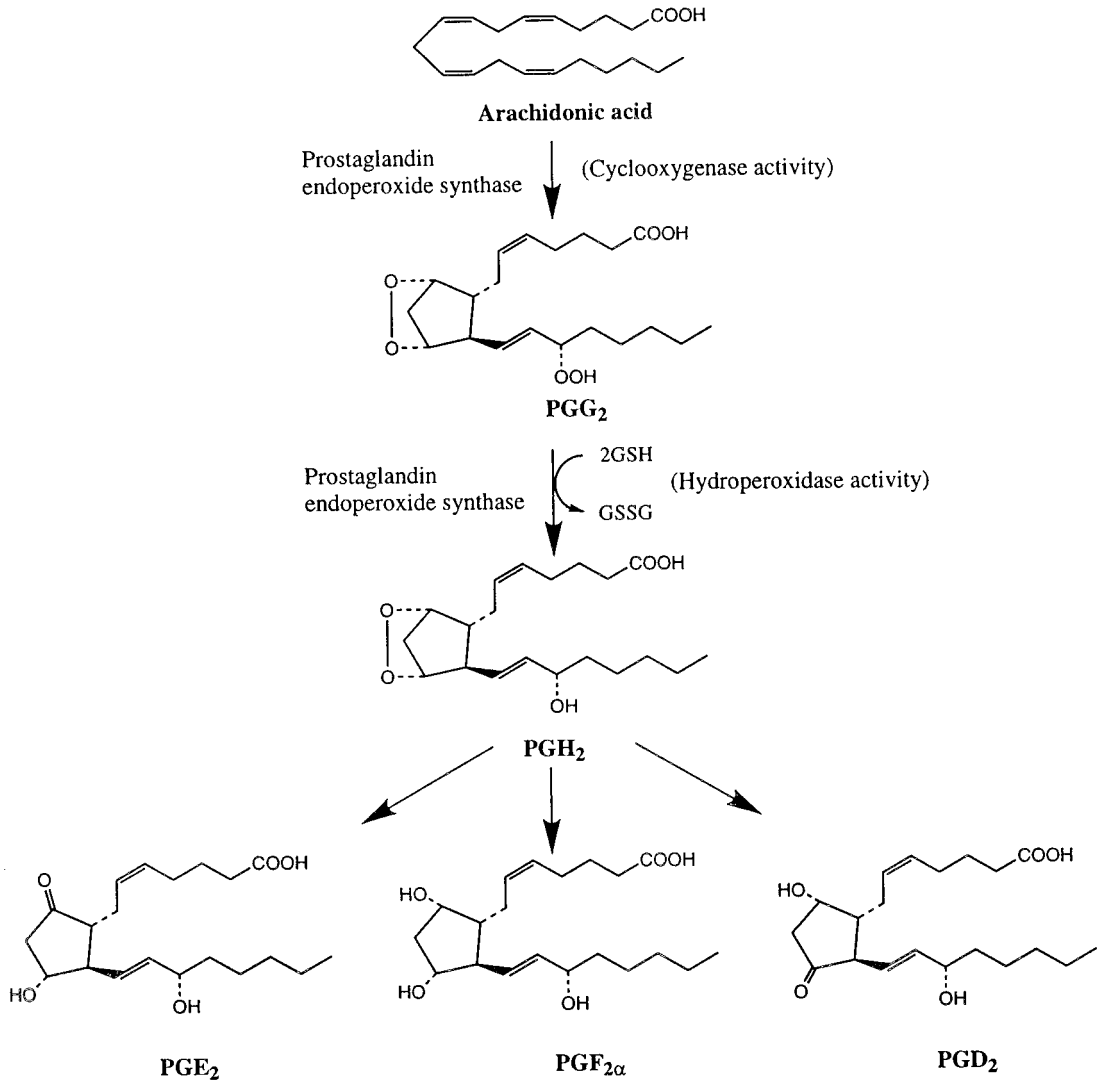

FIG. 6. Biosynthesis of PGE_2, $PGF_{2\alpha}$, and PGD_2 from arachidonic acid.

apparently degraded to form the CysGly and Cys derivatives.

PGD_2-derived compounds are members of a large group of eicosanoids that display cytotoxic activity. A common feature in all these compounds is the presence of a reactive α, β-unsaturated ketone in the cyclopentenone ring. Prostaglandins that lack an α, β-unsaturated ketone are not cytotoxic, and it has been assumed that the α, β-unsaturated ketone is essential for exerting cytotoxic activity (Kato *et al.*, 1986; Bregman *et al.*, 1986; Honn and Marnett, 1985). As illustrated in figure 8, PGD_2 can be transformed to the dehydration product 9-deoxy- Δ^9-PGD_2 (also called prostaglandin J_2), 9-deoxy-Δ^{12}-PGD_2, and 9-deoxy- Δ^9, Δ^{12}-PGD_2 (also called Δ^{12}-prostaglandin J_2; Kikawa *et al.*, 1984; Fitzpatrick and Wynalda, 1983). These compounds exert much greater cytotoxic activity than PGD_2 and most likely are responsible for the cytotoxicity originally ascribed to PGD_2 (Atsmon *et al.*, 1990a; Fukushima *et al.*, 1982; Tanaka *et al.*, 1985; Narumiya and Fukushima, 1985; Kikawa *et al.*, 1984).

α, β-Unsaturated ketones are very susceptible to nucleophilic addition reactions with thiols such as GSH. Atsmon *et al.* (1990b) found that 9-deoxy- Δ^9, Δ^{12}-PGD_2, a potent inhibitor of cell proliferation, and 9-deoxy-Δ^{12}-PGD_2 are conjugated in vitro with GSH to form S-(9-deoxy- Δ^9, Δ^{12}-PGD_2)-GSH conjugate and S-(9-deoxy-Δ^{12}-PGD_2)-GSH conjugate, respectively (fig. 8). After conjugation, further metabolism leads to reduction by the enzyme prostaglandin 11-ketoreductase at the 11-keto and (12–13) moieties to form S-(11-hydroxy-9-deoxy- Δ^9, Δ^{12}-PGD_2)-GSH and S-(11-hydroxy-9-deoxy-Δ^{12}-PGD_2)-GSH (fig. 8). Prior depletion of intracellular GSH decreases the amount of intracellular conjugated prostaglandin and significantly enhances the antiproliferative effect of 9-deoxy- Δ^9, Δ^{12}-PGD_2 on cell growth (Atsmon *et al.*, 1990a). Intracellular GSH also suppresses the binding of 9-deoxy-Δ^9, Δ^{12}-PGD_2 to nuclei,

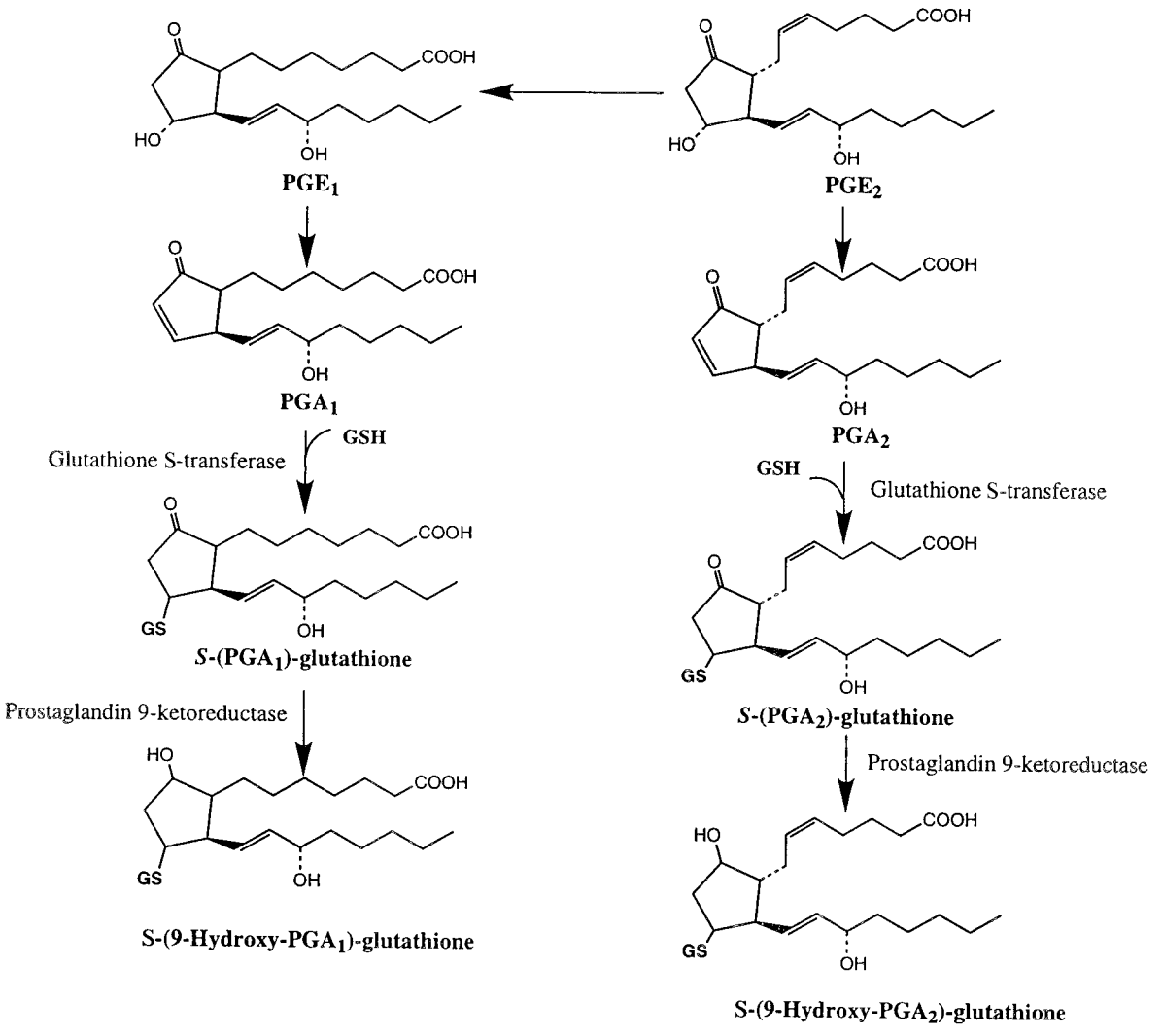

FIG. 7. Conversion of PGE_1 to PGA_1, and PGE_2 to PGA_2, conjugation of PGA_1 and PGA_2 with GSH, and reduction at the keto group to form the respective (9-hydroxyl-PGA)-GSH conjugates.

and inhibits 9-deoxy- Δ^9, Δ^{12}-PGD_2-induced synthesis of heme oxygenase and P67 (a 67-kDa heat shock protein) in porcine aortic endothelial cells (Koizumi *et al.*, 1992). Thus, intracellular GSH may modulate the antiproliferative activity of 9-deoxy- Δ^9, Δ^{12}-PGD_2 and, possibly, of other cytotoxic prostaglandins.

C. Hepoxilins

Hepoxilins are biologically active epoxy alcohols formed from arachidonic acid by initial 12-lipoxygenation and subsequent intramolecular rearrangement of (12S)-hydroperoxy-eicosatetraenoic acid (Pace-Asciak *et al.*, 1983; Pace-Asciak, 1984; fig. 9). There are two position-isomeric hepoxilins isolated, hepoxilin A_3 and hepoxilin B_3. Hepoxilin A_3 is capable of modulating synaptic neurotransmission and neuronal excitability, suggesting a role in neurotransmission (Carlen *et al.*, 1989).

The epoxide moiety of hepoxilins is subject to reaction with epoxide hydrolase or with GSH. As illustrated in figure 9, GSH S-transferase catalyzes the conjugation reaction in which GSH is attached to the 11-carbon position of hepoxilin A_3 to form hepoxilin A_3-C (Carlen *et al.*, 1994; Pace-Asciak *et al.*, 1989, 1990). The biological significance of this GSH conjugation is unknown, although Pace-Asciak *et al.* (1990) and Carlen *et al.* (1994) demonstrated that the GSH conjugate of hepoxilin A_3 causes a hyperpolarization of hippocampal CA1 neurons, once again suggesting a role in neurotransmission.

D. Nitric Oxide

Nitric oxide (NO) is an endogenous reactive intermediate, a free radical, generated by the enzyme NO synthase (Katsuki *et al.*, 1977). NO synthase catalyzes the conversion of L-arginine to L-citrulline and NO, with a 1:1 stoichiometry, and is competitively inhibited by analogues of L-arginine, such as N^G-monomethyl-L-arginine (Palmer *et al.*, 1988; Palmer and Moncada, 1989). The reaction needs NADPH as a reducing cofactor, and an amino acid radical is a likely intermediate (Marletta, 1989). It is now evident that cells other than those in the

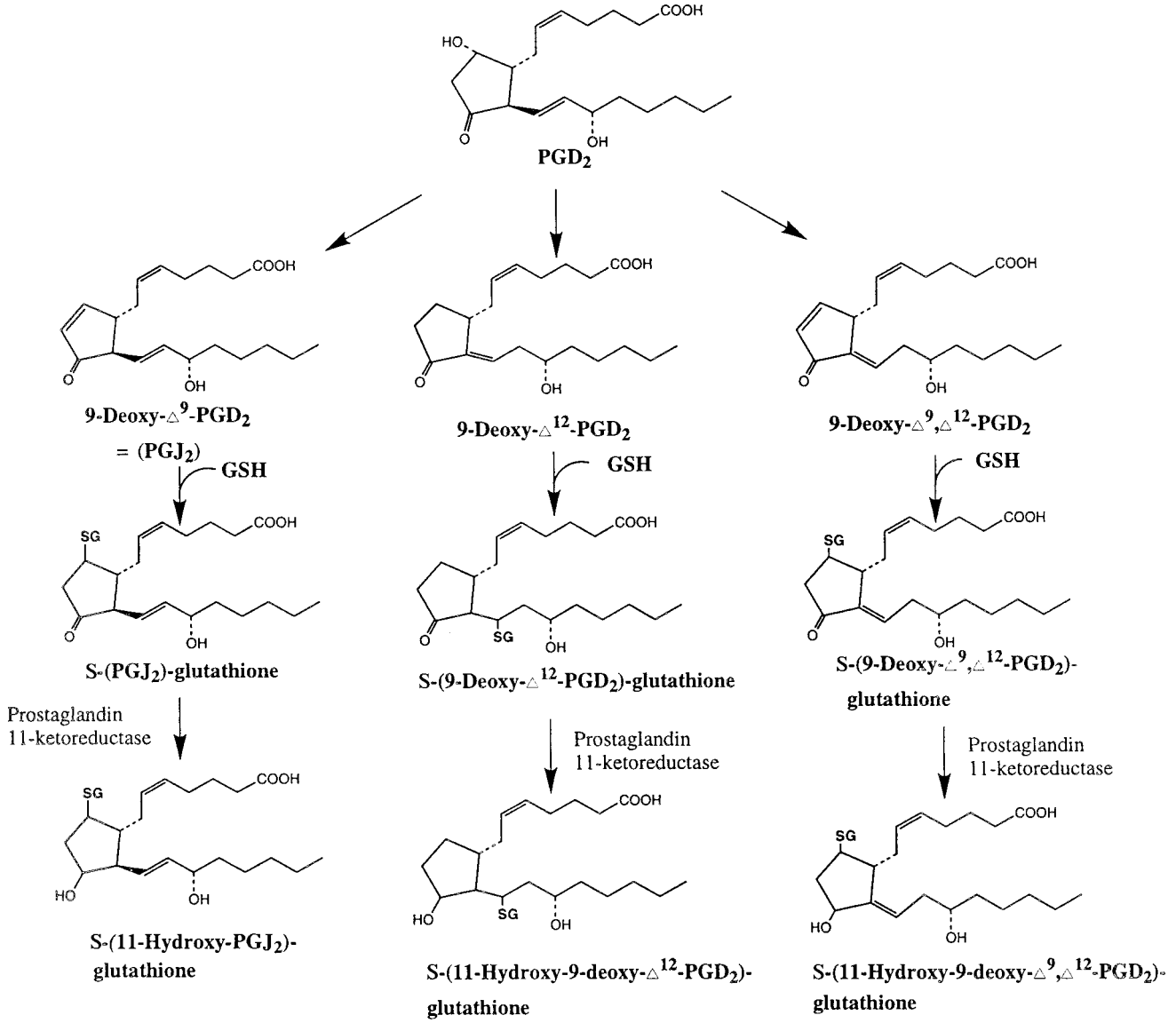

FIG. 8. Conversion of PGD_2 to 9-deoxy-Δ^9-PGD_2 (also called prostaglandin J_2), 9-deoxy-Δ^{12}-PGD_2, and 9-deoxy-Δ^9, Δ^{12}-PGD_2, and the conjugation of these metabolites with GSH.

vascular endothelium, including fibroblasts, macrophages, neutrophils, and neurons, synthesize NO.

NO was identified as the endothelium-derived relaxing factor (EDRF), an endogenous vasodilator (Ignarro, 1990; Palmer *et al.*, 1987). EDRF/NO (*a*) activates a soluble heme-containing guanylate cyclase (Waldman and Murad, 1987); (*b*) stimulates guanosine 3′, 5′-cyclic monophosphate (cGMP) formation; and (*c*) causes vascular smooth muscle relaxation, platelet aggregation inhibition, neurotransmission modulation, and macrophage cytotoxicity (Ignarro, 1990). NO also inhibits neutrophil functions, which include LTB_4 synthesis, superoxide anion (O_2^-) release, and neutrophil adhesion to the vascular endothelium (Ney *et al.*, 1990; Kubes *et al.*, 1991).

In view of the fact that the plasma and cellular milieux contain reactive species that can rapidly inactivate NO, it has been postulated that NO is stabilized by a carrier molecule that preserves its biological activity. Reduced thiol species are candidates for this role, as they readily react in the presence of NO to yield biologically active S-nitrosothiols that are more stable, and possibly more potent, than NO itself (Ignarro *et al.*, 1981; Mendelsohn *et al.*, 1990). Sulfhydryl groups in proteins, and free cysteine and GSH represent an abundant source of reduced thiol in biological systems. There is increasing evidence that at least part of the activity of NO is attributable to S-nitrosothiols derived from the reaction of NO with intracellular thiol compounds like cysteine or GSH (Ignarro, 1990; Myers *et al.*, 1990). S-nitrosothiols may play the same role in the mechanism of action of EDRF as NO; the potent and long-lasting effects of vasodilation and platelet inhibition that they cause are mediated by guanylate cyclase acti-

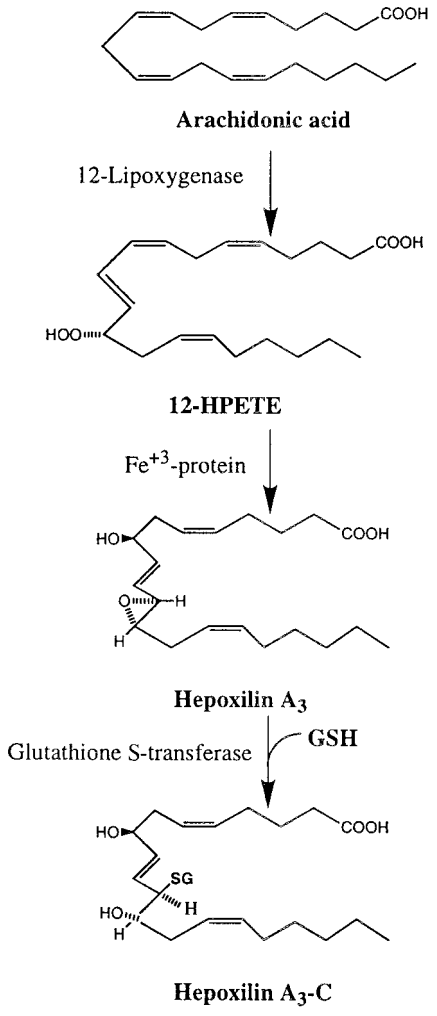

FIG. 9. Formation of hepoxilin A_3 from arachidonic acid, and the GSH conjugation of hepoxilin A_3 to form hepoxilin A_3-C.

vation (Ignarro *et al.*, 1981; Mellion *et al.*, 1983). These observations suggest that S-nitrosothiol groups in proteins may serve as intermediates in the cellular metabolism or bioactivity of NO and that their formation may represent an important cellular regulatory mechanism (Stamler *et al.*, 1992). S-nitrosothiols have also been proposed as biologically active intermediates in the metabolism of organic nitrates (Ignarro *et al.*, 1981; Mellion *et al.*; 1983; Loscalzo, 1985).

S-nitrosoglutathione (GS-NO) has been found endogenously in neutrophils and human airways at micromolar concentrations (Gaston *et al.*, 1993; Clancy *et al.*, 1994). Because GS-NO can yield NO after a one-electron reduction, several investigators have suggested that the physiological role of NO may be mediated by GS-NO and other S-nitrosothiols (Mendelsohn *et al.*, 1990; Armstrong and Stave, 1973). Clancy and Abramson (1992) indicate that neutrophils have the potential to degrade GS-NO and to lower tissue levels of GS-NO, which may limit its biological activity. The degradation of GS-NO by activated neutrophils may contribute to tissue injury at sites of inflammation. Recently Clancy *et al.* (1994) found that NO reacts with intracellular GSH and activates the hexose monophosphate shunt in human neutrophils, providing additional evidence that GS-NO is a bioactive intermediary that may regulate cellular functions. GS-NO also inhibits the sarcoplasmic reticulum-bound creatine kinase (Wolosker *et al.*, 1996), an enzyme that plays a crucial role in the process of energy transduction

NO synthesis is impaired in GSH-depleted human umbilical vein endothelial cells (Ghigo *et al.*, 1993), suggesting that NO synthesis requires GSH and that GSH could play different roles in NO synthesis and its effects, such as (*a*) acting as a reducing cofactor for NO production, in concert with NADPH; (*b*) preventing early inactivation of NO synthase by radical intermediates or NO itself; or (*c*) favoring conversion of NO to GS-NO, which is relatively more stable than NO itself.

The nitrovasodilators amyl and *n*-butyl nitrite have been used as recreational drugs for years, and Meloche and O'Brien (1993) discovered that the NO formed when hepatocytes are incubated with *n*-butyl nitrite mediates many of the cytotoxic effects of this drug, including ATP depletion, lipid peroxidation, and membrane disruption. The formation of GS-NO from alkyl nitrite and GSH may be mediated by the GSH S-transferases (Meyer *et al.*, 1994).

E. Hydroxyalkenals

Lipid peroxidation often occurs in response to oxidative stress, and many kinds of aldehydes are formed when lipid hydroperoxides break down in biological systems. The main process leading to aldehydes is likely to be the β-cleavage of lipid hydroperoxides (Esterbauer *et al.*, 1990; Grosch, 1987). The most intensively studied aldehydes so far are the 4-hydroxyalkenals, in particular, 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE). HNE is an α, β-unsaturated aldehyde that is produced during the oxidation of membrane lipid polyunsaturated fatty acids, such as arachidonic acid and linoleic acid. HHE is formed through both lipid peroxidation (by the degradation of ω 3 polyunsaturated fatty acids) and through nonperoxidative mechanisms, such as the metabolism of the alkaloid senecionine (Segall *et al.*, 1985).

Unlike reactive free radicals, aldehydes are rather long lived and, therefore, can diffuse from their site of origin (i.e., membranes) to reach and attack other targets intracellularly or extracellularly. Numerous biological effects are associated with 4-hydroxyalkenals, including the inhibition of DNA, ribonucleic acid, and protein synthesis; cell proliferation; and the production of various genotoxic effects (Esterbauer *et al.*, 1991).

GSH reacts with 4-hydroxyalkenals to form the structure illustrated in figure 10 (Esterbauer *et al.*, 1975). The initial product is a saturated aldehyde with the GSH residue bound by a thioether linkage at the carbon

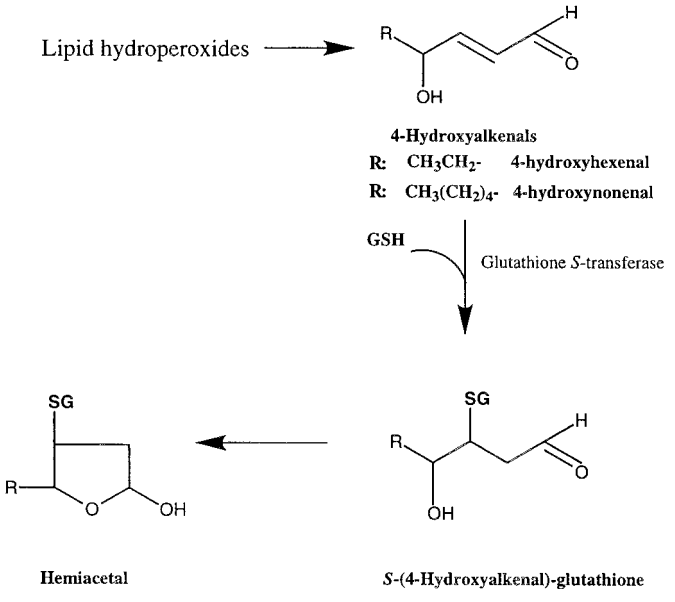

FIG. 10. Conjugation of 4-hydroxyalkenals with GSH.

atom, which then undergoes an intramolecular rearrangement to the five-membered cyclic hemiacetal (fig. 10). One of the primary metabolites of HNE is the GSH S-conjugate (Esterbauer *et al.*, 1991). Grune *et al.* (1994) found that the main products of HNE, as identified in Ehrlich ascites cells, were GSH S-conjugate, hydroxynonenoic acid, and 14-dihydroxynonene, which is consistent with previous findings. GSH S-transferases are the main enzymes involved in the metabolism of these lipid-derived intermediates (Danielson *et al.*, 1987). The enzyme-catalyzed reaction proceeds approximately 300 to 600 times faster than the nonenzymatic reaction. Danielson *et al.* (1987) have proposed that some of the GSH S-transferases may have evolved specifically for the detoxification of HNE and similar aldehydes resulting from lipid peroxidation. The HNE-GSH adduct exhibits a feedback inhibition on the GSH S-transferases. HNE is now used frequently as a substrate to characterize the various types of GSH S-transferases (Tsuchida and Sato, 1990). In rats, the liver was found to have the highest capacity to metabolize HNE (Esterbauer *et al.*, 1985).

HHE also conjugates with GSH (fig. 10). Experiments with injecting tritium-labeled HHE into the portal veins of rats indicate that a part of the radioactivity is excreted in urine as the C-3 mercapturic acid conjugate (Winter *et al.*, 1987). Winter *et al.* (1987) demonstrated that in the whole animal, the HHE-GSH conjugate is exported from the liver and further metabolized to a mercapturic acid. The degradation of HNE to its mercapturic acid can occur in the rat kidney, as well as in other tissues (Petras *et al.*, 1995).

F. Ascorbic Acid

Ascorbic acid and GSH are among the most active reducing substances in living tissues (Meister, 1994; Winkler *et al.*, 1994). Both of these chemicals undergo redox cycling in vivo, and there seems to be a significant interrelationship in this cycling. For example, the toxic effects of GSH deficiency can be prevented by administering ascorbate, indicating that ascorbate has a "sparing" effect on GSH (Meister, 1994). Conversely, GSH seems to be required for the regeneration of ascorbic acid from its oxidized form, dehydroascorbate (Meister, 1994; Winkler *et al.*, 1994). The mechanism for the latter conversion most likely involves a two-electron reduction of dehydroascorbate by GSH, with the intermediate formation of a GSH-ascorbic acid conjugate (fig. 11). The initial step is the nucleophilic addition of GSH to the central carbonyl of dehydroascorbate, followed by reduction, by another GSH molecule, to yield ascorbate and GSSG (fig. 11). Winkler *et al.* (1994) indicate that this nonenzymatic reaction between GSH and dehydroascorbate is the major mechanism for the reduction of dehydroascorbate in mammalian tissues.

G. Dopa and Dopamine

Dopamine is a neurotransmitter derived from the amino acid tyrosine. Tyrosine is hydroxylated to 34-dihydroxyphenylalanine (L-dopa), an immediate precursor of dopamine. This is then decarboxylated to dopamine in a reaction catalyzed by the enzyme aromatic amino acid decarboxylase. Dopamine can be autooxidized to dopamine-*0*-quinones.

As illustrated in figure 12, GSH can conjugate with dopaquinone, dopaminequinone, and methyldopaquinone, which are derived from dopa, dopamine, and methyldopa, respectively; however, the physiological significance of this conjugation is not clear. Glutathionedopa was initially found in malignant melanoma (Agrup *et al.*, 1977) and was thought to be an intermediate in the formation of 5-S-cysteinyldopa. Agrup *et al.* (1977) demonstrated that 5-S-cysteinyldopa, an amino acid that plays a key role in pigment cell metabolism, was formed by conjugation of GSH to dopaquinone (fig. 12), with the subsequent hydrolysis of the peptide by

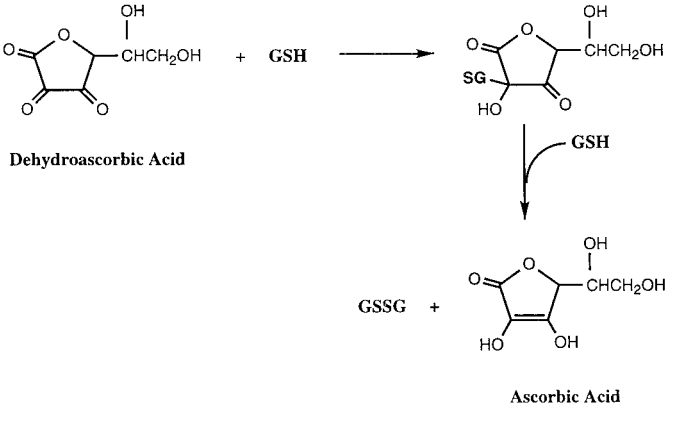

FIG. 11. A proposed mechanism for the two-electron reduction of dehydroascorbic acid by GSH.

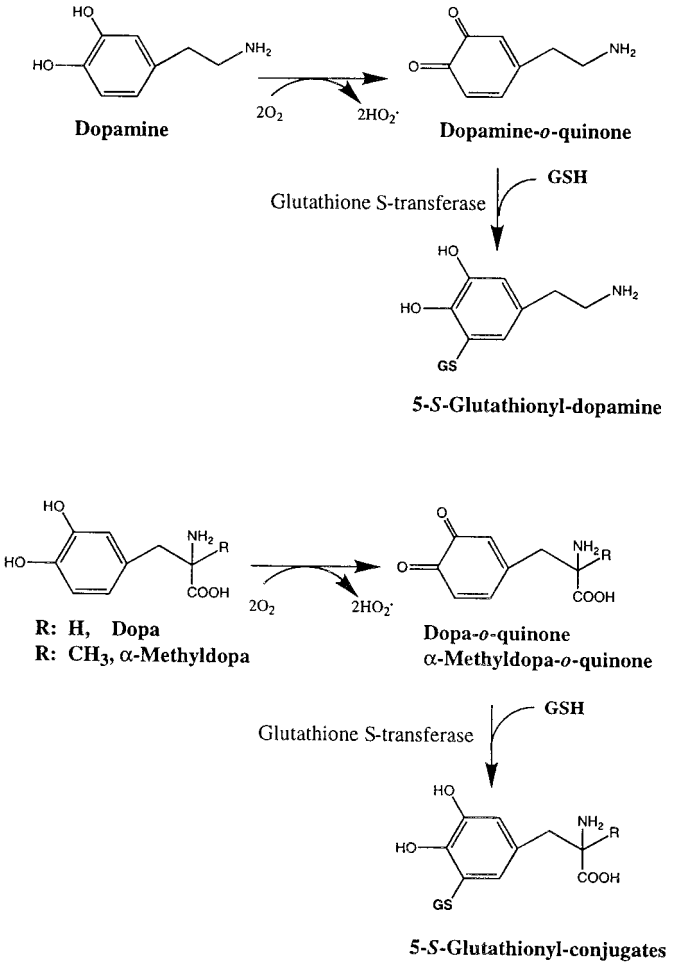

FIG. 12. Conjugation of GSH with dopamine, dopa, and α-methyldopa.

γGT and a dipeptidase. Similar findings by Fornstedt *et al.* (1986) and Palumbo *et al.* (1995) demonstrated the occurrence of 5-S-cysteinyldopa, 5-S-cysteinyldopac, and 5-S-cysteinyldopamine in the brains of all eight mammalian species investigated (i.e., human, rhesus monkey, marmoset monkey, horse, sheep, dog, cat, and rat), indicating that the autooxidation of catechols followed by the coupling to GSH and the attack by peptidases is a normal metabolic pathway in the mammalian brain.

The covalent interaction of quinones with essential protein sulfhydryls may result in enzyme inhibition (Monks and Lau, 1992). Dopamine and its analogs were shown to inhibit melanoma growth by the inhibition of DNA polymerase α, probably by interacting with a sulfhydryl group (Wick, 1980). Ploemen *et al.* (1994) demonstrated that the human GSH S-transferases were inhibited by dopamine, α-methyldopa, and their 5-S-glutathionyl conjugates.

α-Methyldopamine (α-MeDA) is a metabolite of the serotonergic neurotoxicants 34-(±)-(methylenedioxy)-amphetamine (MDA) and 34-(±)-(methylenedioxy)methamphetamine (MDMA; Miller *et al.*, 1995). α-MeDA readily oxidizes, and in the presence of GSH, it forms 5-(S-glutathionyl)-α-methyldopamine (MeDA-SG; Miller *et al.*, 1995). MeDA-SG is metabolized by the brain to 5-(S-cysteinyl)-α-MeDA and 5-(N-acetyl-L-cysteinyl)-α-MeDA, demonstrating that the brain possesses a functional mercapturic acid pathway (Miller *et al.*, 1995). Because all the thiol conjugates of α-MeDA remain susceptible to oxidation, the presence and persistence of these metabolites in brain tissue may contribute to the neurotoxicity of MDA and 34-(±)- (methylenedioxy)methamphetamine. Evidence for this was provided by Miller *et al.* (1996), who showed that intracerebroventricular administration of MeDA-SG to male Sprague-Dawley rats causes behavioral changes identical to those observed after the subcutaneous administration of MDA.

More recently, a novel antibacterial substance from immunized adult S*arcophaga* (flesh fly) was purified; its molecular structure was found to be N-β-alanyl-5-S-glutathionyl-34-dihydroxyphenylalanine, which was synthesized enzymatically from N-β-alanyl-34-dihydroxyphenylalanine (β-Ala-Dopa) and GSH (Leem *et al.*, 1996).

H. Maleic Acid

S-(12-Dicarboxyethyl)GSH (DCE-SG; fig. 13) was isolated from calf lenses in 1963 (Calam and Waley, 1963). It was found in concentrations of 119 nmol per gram of tissues in the rat lens and 72 nmol per gram of tissues in the rat liver (Tsuboi *et al.*, 1990a). In addition, it was found in the rat heart, but not in the rat spleen, kidney, cerebrum, or cerebellum (Tsuboi *et al.*, 1990a).

DCE-SG is synthesized in cells from GSH and L-malate (fig. 13), but the enzyme catalyzing this reaction does not belong to the GSH S-transferase family and is thought to be a novel type of GSH-conjugating enzyme (Tsuboi *et al.*, 1990a).

DCE-SG shows strong inhibitory effects on blood coagulation and platelet aggregation (Tsuboi *et al.*, 1990b), but the mechanism and its significance in vivo is undefined. A possible mechanism for the inhibition of platelet aggregation by DCE-SG may be related to the enhancement of cyclic AMP level and adenylate cyclase activity in platelets produced by this GSH S-conjugate (Tsuboi *et al.*, 1993).

I. Methylglyoxal

GSH can function as a coenzyme in several enzyme-catalyzed reactions, including the glyoxalase reaction (fig. 14). In this reaction, GSH serves as a coenzyme that

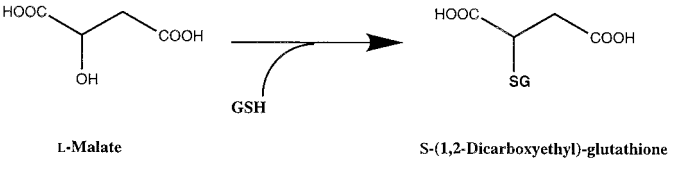

FIG. 13. S-(12-dicarboxyethyl)-GSH synthesis from L-malate and GSH. The enzyme that catalyzes this reaction may not belong to the GSH S-transferase family but may be a novel type of GSH-conjugating enzyme (Tsuboi *et al.*, 1990a).

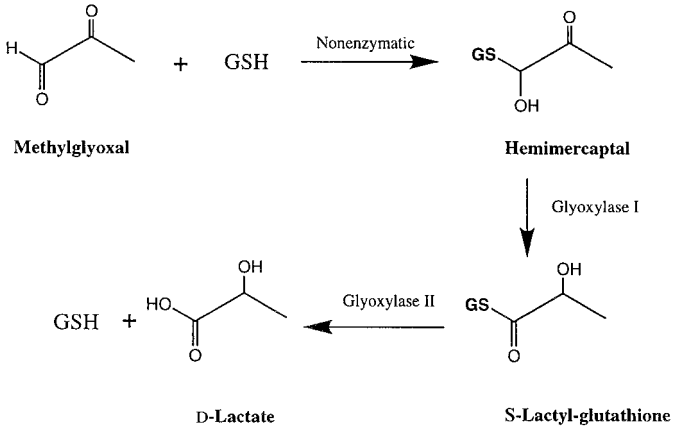

FIG. 14. Glyoxalase reaction: GSH serves as a coenzyme in the conversion of methyglyoxal to D-lactate.

converts methylglyoxal to D-lactate (Behrens, 1941; Racker, 1951; Meister and Anderson, 1983). First, methylglyoxal interacts with GSH nonenzymatically to form the hemimercaptal, the substrate for the reaction catalyzed by glyoxalase I to form S-lactyl-GSH. S-lactyl-GSH is then converted by glyoxalase II into GSH and D-lactate (fig. 14).

The biological significance of this reaction has long been a mystery. Some have suggested that ketoaldehydes play a significant role in the regulation of cell division (Szent-Gyorgyi, 1965; Egyud and Szent-Gyorgyi, 1966). According to this idea, methylglyoxal retards cell growth and glyoxalase promotes cell growth by destroying methylglyoxal, but this has not been definitively demonstrated.

IV. Glutathione Amides: Glutathionylspermidine and Trypanothione

The polyamine spermidine, derived from arginine and methionine, is found in all bacteria and most animal cells. It is a growth factor for some microorganisms and serves to stabilize the membrane structures of bacteria, as well as the structure of ribosomes, some viruses, and the DNA of many organisms.

A GSH-spermidine conjugate, N^1-monoglutathionylspermidine (GspdSH), was initially detected in *Escherichia coli* (*E. coli*)under stationary or anaerobic growth conditions (Tabor and Tabor, 1975; fig. 15). Subsequently, GspdSH and the N^1, N^8-bis(glutathionyl)spermidine conjugate, termed trypanothione, were identified in the pathogenic protozoa of genera *Trypanosoma* and *Leishmania* (Fairlamb *et al.*, 1985; Fairlamb *et al.*, 1986), but not in *E. coli*. These GSH-spermidine conjugates seem to be physiologically important to these organisms. Smith *et al.* (1995) showed that under anaerobic and stationary-phase conditions, 80% of the total GSH in *E. coli* is in the form of GspdSH, suggesting that GspdSH may be a better DNA-protectant against radical- or oxidant-induced damage than GSH. Some think that parasites maintain redox balance and defend

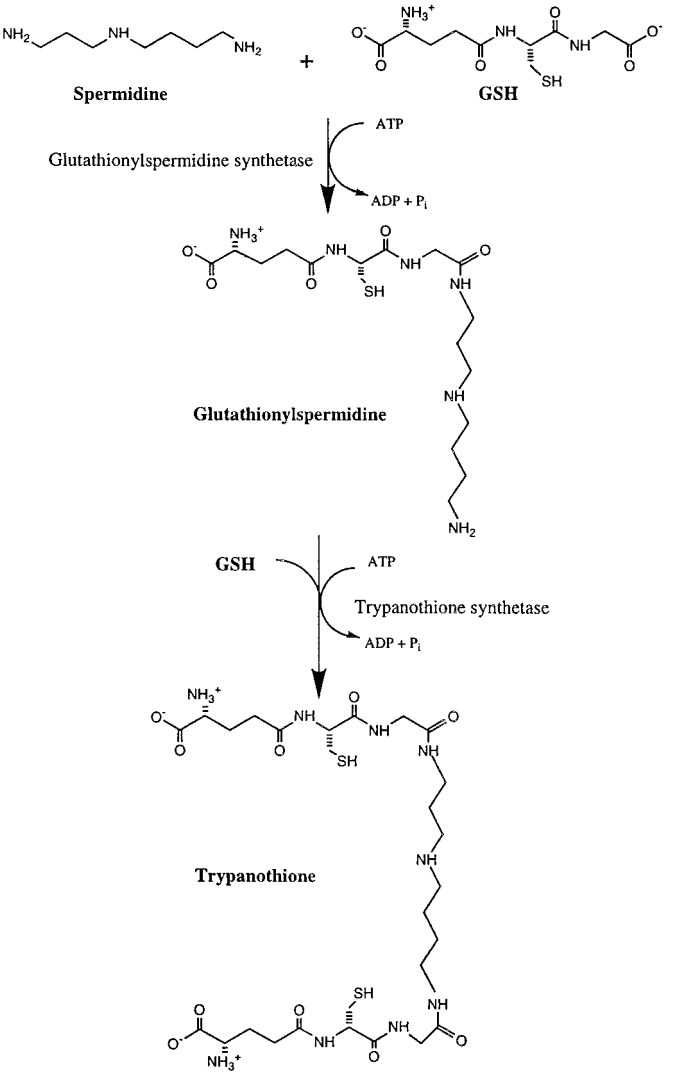

FIG. 15. Biosynthesis of trypanothione in bacterial systems. Conjugation of the glycine carboxyl of GSH and the first amino group of spermidine is catalyzed by glutathionylspermidine synthetase. Trypanothione synthetase then catalyzes the addition of a second molecule of GSH to glutathionylspermidine to form trypanothione.

against oxidant stress by synthesizing trypanothione, because the parasites lack typical catalase and GSH peroxidase-GSSG reductase enzyme couples (Bollinger *et al.*, 1995). Therefore, trypanothione metabolism has been considered a possible target for new antiparasitic drugs (Fairlamb *et al.*, 1985).

Glutathionylspermidine synthetase was initially partially purified from *E. coli* (Tabor and Tabor, 1966). Recently, Bollinger *et al.* (1995) purified *E. coli* glutathionylspermidine synthetase to near homogeneity, and the gene encoding it was isolated and sequenced. As illustrated in figure 15, glutathionylspermidine synthetase couples ATP hydrolysis with the formation of an amide bond between spermidine and the glycine carboxylate of GSH. This enzyme was also found to have a second catalytic activity, glutathionylspermidine amide bond hydrolysis. Thus, the bifunctional glutathionyl-

spermidine synthetase/amidase catalyzes opposing amide bond-forming and cleaving reactions, with a net hydrolysis of ATP. Trypanothione synthase then catalyzes the addition of the second GSH molecule to the free primary amine of glutathionylspermidine to form trypanothione. The lack of the trypanothione in *E. coli* seems to be because of the absence of trypanothione synthase (Smith *et al.*, 1995).

V. Glutathione Thioesters

It has been discovered that many enzymes are activated or inhibited in vitro by the disulfide exchange between the protein and small-molecule disulfides. The fundamental equation for a thiol/disulfide exchange is:

$$R'SH + RSSR \rightleftharpoons R'SSR + RSH$$

where R and R′ are either cysteine, cysteine-containing peptides, or other sulfhydryl-containing compounds.

The thiol/disulfide ratio seems to be of great importance in the regulation of cellular metabolism, and GSH plays the key role in regulating the thiol/disulfide exchange (Gilbert, 1982).

Many enzymes are activated by GSSG, including glucose-6-phosphatase, acid phosphatase, γ-aminolaevulinate synthetase, and fructose 16-bisphosphatase (Gilbert, 1982; Ondarza, 1989). Enzymes that are inhibited by GSSG include glycogen synthetase D, pyruvate kinase, adenylate cyclase, phosphorylase phosphatase, ribonucleotide reductase, phosphofructokinase, glycogen debranching enzyme, fatty acid synthase, and 15 OH-prostaglandin dehydrogenase. It has been reported that GSSG possesses the ability to stimulate microsomal GSH S-transferase activity (Nishino and Ito, 1989) and to cause the sensitization of calcium release to inositol 14,5-trisphosphate in permeabilized hepatocytes (Renard *et al.*, 1992).

Similar processes are involved for coenzyme A and the GSH-coenzyme A mixed disulfide (CoASSG). CoASSG was initially identified in the rat (Ondarza and Aubanel, 1960; Ondarza, 1965) and bovine liver (Wilken and Hansen, 1961; Chang and Wilken, 1965). Numerous reports indicate that these sulfur compounds are normal components of both eukaryotic cells and many eubacteria, and that they play an important role in enzyme regulation. For instance, CoASSG was found to inhibit GSSG reductase (Ondarza, 1966), phosphofructokinase (Gilbert, 1982), and fatty acid synthase (Walters and Gilbert, 1986). In contrast, fructose 16-bisphosphatase is activated by CoASSG (Nakashima *et al.*, 1969).

Intracellular GSSG levels rise during hydroperoxide metabolism, and these perturbations of GSH status are accompanied by significant metabolic changes that are mediated by the formation of mixed disulfides between GSSG and thiol groups present in proteins (enzymes) or low molecular weight compounds (Akerboom *et al.*, 1982, 1984; Kosower and Kosower, 1978; Crane *et al.*, 1982, 1983). For example, Crane *et al.* (1982) found that there is a rise of CoASSG during hydroperoxide metabolism in perfused rat liver but that coenzyme A disulfide levels are virtually unchanged. Thus, as a result of the metabolism of hydroperoxides, a sulfhydryl exchange occurs between the elevated levels of GSSG and cellular coenzyme A, with the formation of CoASSG as the major component. Crane *et al.* (1983) also found that there is a decreased flux through pyruvate dehydrogenase by thiol oxidation during *t*-butyl hydroperoxide metabolism in the perfused rat liver. Akerboom *et al.* (1984) demonstrated that the addition of hydrogen peroxide inhibits the endogenous release of bile acids. More recently, Schluter *et al.* (1995) demonstrated that a potent vasoconstrictor isolated from bovine adrenal glands was CoASSG. At a concentration of 10^{-12} mol/L, CoASSG increases renal vascular resistance. Intra-aortic injection of 5×10^{-10} mol CoASSG increases blood pressure in the intact animal. Besides its vasopressor properties, this substance potentiates the effects of angiotensin II on vascular tone (Schluter *et al.*, 1995).

VI. Glutathione Mercaptides

In contrast to the majority of organic chemicals that undergo metabolic degradation, metal elements are indestructible in biological tissues. Once incorporated into an organism, their physiological and toxicological effects are regulated by two general mechanisms: binding to specific ligands and excretion. Binding to amino acids, peptides, proteins, phospholipids, and other tissue constituents modulates both their reactivity (toxicity) and biological effects. Because most heavy metals are present in biological tissues as complexes with specific ligands rather than as free cations (the form usually attributed to metal toxicity), these ligands play a critical role in metal homeostasis (Ballatori, 1994).

Among the metal binding ligands, GSH is one of the most versatile and pervasive. GSH contains six potential coordination sites for metal binding: the cysteinyl sulfhydryl, the glutamyl amino, the glycyl and glutamyl carboxyl groups, and the two peptide linkages. Of these, the sulfhydryl residue exhibits the highest affinity for several metals, including mercury, cadmium, copper, zinc, silver, arsenic, and lead (Ballatori, 1994). A metal bound to the sulfhydryl group of GSH can be stabilized by coordination with one of the other potential binding sites within the tripeptide. In general, more stable structures are obtained when these divalent metals form a 1:2 (metal:sulfhydryl) complex, to generate either GS-M-SG, GS-M-SR or RS-M-SR', where RS is an additional ligand, M is metal, and GS is glutathione moiety. The chemistry of monovalent heavy metal compounds (M^+) is more straightforward, generally forming 1:1 complexes (Martell and Calvin, 1952; Lenz and Martell, 1964).

The formation of GSH mercaptides is highly favored thermodynamically and occurs spontaneously (nonenzy-

matically) under physiological conditions (Ballatori, 1994). The reaction is reversible, but the equilibrium is shifted toward the formation of the weakly dissociating mercaptides. Despite the thermodynamic stability of these metal-sulfhydryl complexes, they are generally kinetically labile. That is, the metal rapidly exchanges between available sulfhydryl ligands. This property is the key to the rapid mobility of metals in biological systems (Clarkson, 1972, 1993).

GSH modulates the disposition and toxicity of metals by at least four mechanisms: (*a*) it helps in the mobilization and delivery of metals between ligands; (*b*) it transports metals across cell membranes as GSH complexes; (*c*) it serves as a source of cysteine, an amino acid that plays a central role in metal homeostasis; and (*d*) it serves as a cofactor for redox reactions, yielding metal compounds with different speciation or biochemical forms (Ballatori, 1994). The following discussion will focus on the endogenous metals copper and selenium.

A. Copper

GSH is responsible for the mobilization and delivery of copper during the biosynthesis of copper proteins. GSH maintains the intracellular pool of free copper in the Cu(I) oxidation state by providing reducing equivalents for the conversion of Cu(II) to Cu(I) (Freedman *et al.*, 1989). In the Cu(I) oxidation state, copper forms stable GSH complexes (Ciriolo *et al.*, 1990), and these complexes serve as a source of Cu(I) for incorporation into apometallothionein (Freedman *et al.*, 1989), aposuperoxide dismutase (Steinkuhler *et al.*, 1991), apohemocyanin (Brouwer and Brouwer-Hoexum, 1992), and phytochelatins (Mehra and Mulchandani, 1995).

Using a hepatoma cell line, Freedman *et al.*, (1989) demonstrated that more than 60% of the copper in the cytosol of the wild-type and a copper-resistant cell line was bound to GSH. The resistant cells had nearly four times the concentration of GSH than the wild type and had highly elevated levels of metallothionein. Resistance to the toxic effects of copper was correlated with intracellular GSH concentration. These investigators demonstrated that intracellular Cu(I) was transferred from GSH to metallothionein in a reversible reaction. Formation of the Cu(I)-metallothionein complex required the presence of the Cu(I)-GSH complex and was not readily achieved in its absence. It was suggested that the transfer of copper from GSH to metallothionein occurred via a GSH-Cu-metallothionein intermediate. Similarly, Ciriolo *et al.* (1990) demonstrated that the Cu(I)-GSH complex serves as the substrate for introducing copper into CuZn-superoxide dismutase. GSH complexes of Cu(I) but not Cu(II) were able to reconstitute enzyme activity.

In addition, Brouwer and Brouwer-Hoexum (1992) demonstrated that the Cu(I)-GSH complex, but not the Cu(I)-metallothionein complex, is able to serve as a source of Cu(I) in the in vitro reconstitution of lobster apohemocyanin. Although metallothioneins have long been implicated in the delivery of copper for the biosynthesis of metalloproteins (Harris, 1991), Brouwer and Brouwer-Hoexum's (1992) results indicate that the metallothionein complex is not able to accomplish this task for apohemocyanin. Thus, GSH not only acts to deliver copper to metallothionein during its biosynthesis (Freedman *et al.*, 1989), but when the cellular supply of copper is limited, GSH can mobilize the copper from the metallothionein for delivery and incorporation into apometalloproteins. In this case, metallothionein apparently serves as a temporary storage area for cytoplasmic copper, whereas GSH serves both as a vehicle for the delivery of copper to metallothionein and as a vehicle for removing the metal from metallothionein in times of need. In contrast, metallothionein plays a more important role in the long-term detoxification of high concentrations of copper (Harris, 1991). Metallothionein levels directly reflect intracellular copper concentrations (Sone *et al.*, 1987).

The binding of copper to albumin, a major copper-binding protein in blood plasma, also involves GSH. Suzuki *et al.*, (1989) demonstrated that cupric ions injected intravenously into rats were gradually incorporated into an albumin-Cu-cysteine or albumin-Cu-GSH complex. These albumin complexes constitute the largest labile pool of plasma copper and probably play a key role in the distribution and cellular uptake of this metal.

Recently Musci *et al.* (1996) demonstrated that the Cu(I)-GSH complex efficiently acted in vitro as the source of Cu(I) in the reconstitution of apoceruloplasmin. Ceruloplasmin is an α_2-glycoprotein in the plasma of all vertebrates. The Cu(I)-glutathione complex seems the most suitable source of copper to reestablish the native structural and functional properties of ceruloplasmin at neutral hydrogen ion concentration (pH).

GSH is also involved in the transport of copper as GSH-Cu(I) complex. Some believe that GSH plays a role in the efflux of copper from liver cells into bile because of the observation that hepatic GSH depletion leads to an inhibition of the biliary transport of copper (Alexander and Aaseth, 1980; Nederbragt, 1989; Houwen *et al.*, 1990). Mutant Wistar rats that are deficient in their ability to secrete GSH into bile also have a diminished ability to transport excess copper into bile (Houwen *et al.*, 1990). In contrast, the basal excretion of endogenous copper is unaffected in the mutant Wistar rats.

In addition, GSH plays a part in the reduction of Cu(II) to Cu(I), the form incorporated into apometalloproteins, as discussed earlier in Section VI.A. Cu(I) forms a relatively stable GSH complex and, as such, is more energetically favored to exchange with, and bind to, internal metal-binding sites of metalloproteins.

B. Selenium

GSH is involved in the reductive detoxification of selenite (Ganther, 1966) but may also promote the forma-

tion of toxic seleno compounds (Vernie *et al.*, 1979; Frenkel and Falvey, 1989). The selenite-mediated inhibition of protein and nucleic acid synthesis is potentiated by the addition of GSH, but the mechanism is not well defined (Vernie *et al.*, 1979; Frenkel and Falvey, 1989). The reaction of GSH with selenite (SeO_3^{-2}) produces selenodiglutathione, GSH selenopersulfide, elemental selenium, and selenide.

Selenium decreases the toxicity of methylmercury in experimental animals by promoting the conversion of methylmercury to a biologically less active form, bis-(methylmercuric)selenide. GSH facilitates the reduction of selenium to a chemical form (probably H_2Se) that can react with methylmercury to yield bis(methylmercuric)-selenide (Iwata *et al.*, 1981).

VII. Summary

A large number of endogenous compounds are able to conjugate with GSH. The binding of GSH to endogenous compounds is known to serve important biological functions besides detoxification. GSH conjugation serves to limit and regulate the reactivity of the chemicals; it facilitates their membrane transport and elimination from the cell and organism; and in many cases, it leads to the formation of essential biological intermediates. Some of the endogenous GSH conjugates have not been well characterized, and it is likely that there are many others that have yet to be discovered.

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