GLUTATHIONE METABOLISM AND ITS ROLE IN HEPATOTOXICITY

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Abstract—Glutathione (GSH) fulfills several essential functions: Detoxification of free radicals and toxic oxygen radicals, thiol-disulfide exchange and storage and transfer of cysteine. GSH is present in all mammalian cells, but may be especially important for organs with intense exposure to exogenous toxins such as the liver, kidney, lung and intestine. Within the cell mitochondrial GSH is the main defense against physiological oxidant stress generated by cellular respiration and may be a critical target for toxic oxygen and electrophilic metabolites. Glutathione homeostasis is a highly complex process, which is predominantly regulated by the liver, lung and kidney.

CONTENTS

1. THE STRUCTURE OF GLUTATHIONE

Glutathione is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine), that serves several essential functions within the cell. It is present in millimolar concentrations intracellularly and is therefore the main non-protein thiol in almost all aerobic species. Under physiological conditions glutathione reductase will rapidly reduce any oxidized glutathione (GSSG) to the reduced, thiol form (GSH), so that more than 98% of intracellular glutathione is GSH. The rest is present within the cell as the oxidized, disulfide form (GSSG), as mixed disulfides (mainly GSS-protein), and as thioethers.

Two aspects of the structure of glutathione directly determine the enzymatic pathways capable of metabolizing it (Fig. 1). Most peptides can be cleaved by peptidases at the α-carboxyl peptide linkage of the N-terminal amino acid. However in glutathione the N-terminal glutamate and cysteine are linked by the γ-carboxyl group of glutamate, restricting cleavage to γ-glutamyltranseptidase, which occurs on the external surface of certain cell membranes. Glutathione is therefore resistant to intracellular degradation and can only be cleaved by cell types that have γ-glutamyltranspeptidase on the cell membrane. The presence of the C-terminal glycine protects the peptide against cleavage by intracellular γ-glutamylcyclotransferase, further restricting the route by which it can be catabolized.

The keystone to the functions fulfilled by GSH is cysteine, which provides the reactive thiol group. These functions include: (1) Detoxification of...
exogenous and endogenous compounds such as reactive electrophiles and toxic oxygen metabolites (peroxides); (2) maintaining essential thiol status of proteins and other molecules; (3) providing the major molecular form by which cysteine can be stored within the organism and used for transfer between organs. These functions will be described in the subsequent section. However, it should be emphasized that glutathione serves multiple other functions such as a role in leukotriene and prostaglandin metabolism, reduction of ribonucleotides to deoxyribonucleotides and modulation of microtubule-related processes.

2. FUNCTIONS OF GLUTATHIONE

2.1. DETOXIFICATION

2.1.1. Conjugation of Electrophiles

Many chemicals in the environment are not hazardous in the naturally occurring form, but rather are metabolized within the body to unstable electrophilic metabolites which are the ultimate toxic or carcinogenic moieties (Heidelberger, 1975; Miller, 1978; Gelboin, 1980). The cytochrome P-450-mediated mixed-function oxygenase system may be the most important pathway for the formation of such reactive metabolites (Gelboin, 1980; Conney, 1982; Okey and Nebert, 1983). Glutathione plays a major role in detoxifying many reactive metabolites by either spontaneous conjugation or by a reaction catalyzed by GSH-S-transferase. The GSH conjugate is then attacked by y-glutamyltransferpeptidase which removes the y-glutamyl moiety, leaving a cysteinyl–glycine conjugate. The cysteiny1–glycine bond is cleaved by dipeptidase, resulting in a cysteiny1 conjugate. Finally, a mercapturic acid is formed by the acetylation of cysteine. The metabolism of GSH conjugates to mercapturic acid begins in either the biliary tree or intestine or in the kidney, but the formation of the N-acetylcysteine conjugate usually occurs in the kidney.

The metabolism of leukotrienes is virtually the same as that of exogenous compounds conjugated to GSH. Some cytosolic GSH-S-transferase isozymes (Mannervik et al., 1984; Söderström et al., 1985; Tsuchida et al., 1987) and a microsomal isozyme (Bach et al. 1984; Yoshimoto et al., 1985) catalyze the conversion of leukotriene A4 to leukotriene C4, a glutathione conjugate. Removal of glutamate by y-glutamyltransferpeptidase results in the cysteinyl–glycine conjugate leukotriene, D4. This in turn is metabolized by dipeptidase forming the cysteiny1 conjugate, leukotriene E4.

Like most phase II reactions, conjugation to GSH is almost always a detoxifying pathway. However, there are several instances described in which this pathway actually results in the formation of reactive metabolites. For example, the GSH conjugate of dibromoethane transforms to a 2-bromo-thioether, which then is converted to a highly reactive intermediate, possibly an episulfonium (Rannug, 1980; Van Bladeren et al., 1981; Rannug et al., 1978). This compound is highly toxic (Wong et al., 1982; Storer and Conolly, 1983), mutagenic in a variety of test systems (Storer and Conolly, 1983; Rosenkranz, 1977; Rannug, 1980; Van Bladeren et al., 1981; White et al., 1981), and carcinogenic in rodents (Wong et al., 1982; Huff, 1983). The role of GSH activation of dibromoethane has been examined in GSH-deficient fibroblasts taken from patients with an inborn error of GSH metabolism (GSH-synthetase deficiency). Dibromoethane induced significantly less genotoxic damage in the GSH-deficient fibroblasts than in normal human fibroblasts (DeLeve, 1988). This suggests that the range of GSH levels seen in humans is large enough to modify the mutagenic effect of dibromoethane and demonstrates how genetic variation of GSH levels could impact on the risk of cancer.

2.1.2. Anti-oxidant Function

All aerobic organisms are subject to a physiological oxidant stress as a consequence of aerobic metabolism. The intermediates which are formed, such as superoxide and hydrogen peroxide, lead to the further production of toxic oxygen radicals that can cause lipid peroxidation and can disrupt metabolic processes. Through a reaction catalyzed by GSH peroxidase, glutathione is the predominant defense against these toxic products of oxygen metabolism. As will be discussed in a later section, glutathione sequestered within the mitochondria is particularly important in the defense against both physiologically and pathologically generated oxidant stress.

Endogenously produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase (Fig. 2). As a consequence GSH is oxidized to GSSG, which in turn is rapidly reduced back to GSH by GSSG reductase at the expense of NADPH, thereby forming a closed system (redox cycle). The reduction of organic

![Fig. 1. The structure of the tripeptide glutathione. Reproduced from DeLeve and Kaplowitz (1990), with permission of the copyright holder, Thieme-Stratton, Inc., New York.]
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**FIG. 2. Anti-oxidant pathways.** Hydrogen peroxide is metabolised by GSH peroxidase in the cytosol and by catalase in the peroxisome. Organic peroxides can be reduced by either GSH peroxidase or GSH S-transferases of the alpha class. Reproduced from DeLeve and Kaplowitz (1990), with permission of the copyright holder, Thieme-Stratton, Inc., New York.

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 alpha class. Patients with inborn errors of GSH metabolism that result in diminished intracellular GSH, such as \( \gamma \)-glutamylcysteine synthetase deficiency and GSH synthetase deficiency, demonstrate symptoms due to oxidant stress. One of the manifestations of both of these hereditary enzyme deficiencies is chronic hemolytic anemia due to the inability to combat physiological oxidant stress. An example of an acquired deficiency of GSH is acquired immune deficiency syndrome (AIDS) (Buhl et al., 1989). Clinical trials are currently underway to assess whether correcting this acquired GSH-deficiency will improve the immunologic and brain dysfunctions found in these patients.

To maintain the redox state of the cell, the GSSG generated during oxidant stress must be reduced by GSSG reductase at the expense of NADPH. In most cell types NADPH is produced in the mitochondria and cytosol. However erythrocytes lack mitochondria and in this cell type NADPH is only formed in the cytosol. The cytosolic reaction which produces NADPH is the oxidation of glucose-6-phosphate to 6-phosphogluconate, a reaction that is part of the hexose monophosphate shunt. Thus in G-6-PD deficiency, one of the most prevalent genetic defects, the formation of NADPH in erythrocytes is impaired. This results in mild chronic hemolysis and severe acute hemolysis under oxidant stress. This is another example of the essential role played by an intact system for reducing GSSG.

Under severe oxidant stress the ability of the cell to reduce GSSG by GSSG reductase may be overcome, leading to accumulation of GSSG within the cytosol. To protect the cell from a shift in the redox equilibrium, the cell can actively transport GSSG out of the cell (Fig. 2) as has been demonstrated in erythrocytes (Srivastava and Beutler, 1969a; Srivastava et al., 1974; Kondo 1980), eye (Srivastava and Beutler, 1969b), heart (Ishikawa and Sies, 1984) and liver (Sies et al., 1972, 1978; Akerboom et al., 1982; Adams et al., 1983; Lauterburg et al., 1984b).

Given the above-mentioned ability of cells to export GSSG, it is not surprising that plasma GSSG seems to correlate well with shifts in the intracellular redox state. Increases in plasma GSSG parallel the increases in GSSG found in rat and human liver and skeletal muscle during vigorous exercise (Lew et al., 1985; Gohil et al., 1988). Subtle differences in the redox equilibrium of tissues are also measurable, as demonstrated by the doubling of plasma GSSG found in rabbit venous blood as compared to the arterial GSSG concentration (Costagliola et al., 1988).

In addition to reduction by GSH peroxidase, the cell can also degrade hydrogen peroxide by catalase (Fig. 2). This enzyme is compartmentalized in the peroxisome (Jones, 1982; Eklöw et al., 1984) and has a high \( K_m \) for hydrogen peroxide. As a result of these two factors, catalase plays a limited role in comparison to the anti-oxidant function of GSH. However, catalase may be of greater importance under conditions where NADPH is a limiting factor or hydrogen peroxide levels are particularly high (Jones et al., 1981).
2.2. THIOL–DISULFIDE EXCHANGE

When life on this planet first appeared the atmosphere exhibited very low oxygen tension. Intracellular sulfhydryl groups in proteins developing under those conditions would have been in the reduced, thiol form rather than in the oxidized, disulfide form. As oxygen tension in the environment increased, many existing cell functions would have been disrupted without a system to preserve low oxygen tension within the cell and to prevent or reverse oxidation of essential thiols (Fahey, 1977). The system which evolved to fulfill this function was thiol–disulfide exchange by glutathione and catalyzed by thiol–transferase. The equation for this reaction is:

\[ \text{protein} - \text{SSG} + \text{GSH} \rightleftharpoons \text{protein} - \text{SH} + \text{GSSG}. \]

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, as described above, others require oxidized, disulfide, groups. Since the thiol-transferase reaction is bidirectional, the equilibrium will be determined by the redox state of the cell. Small disulfides such as GSSG or cysteine can react with cysteine moieties within enzymes to form protein-mixed disulfides. This reaction would activate enzymes that have essential disulfide groups.

The effect of thiol–disulfide status on enzyme activity has been particularly well studied for the enzymes involved in glucose metabolism (Ziegler, 1985), such as glycogen phosphorylase phosphatase (Gratecos et al., 1977; Shimazu et al., 1978; Usami 1980), glycogen synthetase (Ernest and Kim, 1973), phosphofructokinase (Froede et al., 1968; Gibert, 1982), fructose-1,6-diphosphatase (Pontremoli et al., 1967, 1982; Nakashima et al., 1969; Pontremoli and Horecker, 1970; Nakashima and Horecker, 1970; Moser et al., 1982), hexokinase (Redkar and Kenkare, 1972), pyruvate kinase (Van Berkel et al., 1973; Mannervik and Axelsson, 1980). Thus in vitro studies have shown that several key enzymes are activated by changes in protein thiol status, but further studies will need to confirm that this also occurs in vivo.

Oxidation of cysteine groups enhances glycogen catabolism and inhibits synthesis of glycogen. Glycogenolysis by glycogen phosphorylase A occurs when glycogen phosphorylase phosphatase is inactivated by oxidation of essential cysteine moieties (Gratecos et al., 1977; Shimazu et al., 1978; Usami et al., 1980). Conversely, glycogen synthesis is inhibited when disulfides react with glycogen synthetase (Ernest and Kim, 1973).

Regulation of glucose metabolism must be particularly well controlled when enzymes catalyze directly opposing reactions. Phosphofructokinase catalyzes a specific glycolytic reaction, whereas fructose-1,6-diphosphatase catalyzes the same reaction in the opposite, gluconeogenic, direction. Ongoing activity of both of these enzymes would set up a futile cycle, that would deplete ATP without any metabolic benefit (Lehninger, 1982). Experimental evidence suggests that small disulfides may contribute to the inhibition of phosphofructokinase (Froede et al., 1968; Gilbert, 1982), whereas fructose-1,6-diphosphatase may be activated by disulfide formation (Pontremoli et al., 1967, 1982; Nakashima et al., 1969; Pontremoli and Horecker, 1970; Nakashima and Horecker, 1970; Moser et al., 1982).

The regulatory enzymes of cholesterol metabolism may also be influenced by thiol–disulfide status. 3-Hydroxymethylglutaryl-Co-A reductase catalyzes the rate-limiting step for cholesterol biosynthesis: The conversion of 3-hydroxymethylglutaryl-Co-A to mevalonate. Several factors are involved in the regulation of this enzyme, including inhibition by the end-product, cholesterol. Disulfide formation may be one of these regulatory mechanisms (Kawachi and Rudney, 1970; Gilbert and Stewart, 1981; Dotan and Schechter, 1982; Roitelman and Schechter, 1984).

2.3. STORAGE AND TRANSFER OF CYSTEINE

Cysteine cannot be transported in the plasma or stored within the cell as simple cysteine, since it would rapidly auto-oxidize to cystine, producing potentially toxic oxygen radicals. The preponderance of the oxidized form is demonstrated by the high ratio (14:1) of cystine to cysteine in rat plasma (Lash and Jones, 1985). This auto-oxidation reaction is avoided by storing almost all non-protein cysteine as GSH (Fig. 3).

Glutathione is synthesized in all mammalian cells, but most of the turnover occurs in the liver and kidney. Breakdown of plasma GSH occurs...
at the external surface of cells exhibiting high \( \gamma \)-glutamyltranspeptidase and dipeptidase activities. The constituent amino acids of GSH can be taken up in the same organ from which GSH was released (intraorgan homeostasis) or GSH can escape and transfer via plasma to another organ for breakdown (interorgan homeostasis). Glutathione synthesized in the liver is released into bile and plasma and is almost the sole source of plasma GSH. Studies done in rats have shown very little breakdown of plasma GSH by the liver, so that the plasma GSH is broken down and utilized by other organs. In the kidney GSH is released into the tubules. Under normal conditions GSH is broken down to its constituent amino acids in the tubules, so that virtually no GSH appears in the urine.

The function of glutathione as the main tissue and plasma storage form of cysteine has been demonstrated experimentally. In fasting rats there is an increased peripheral requirement for cysteine and a consequent fall in plasma GSH. Under these conditions hepatic synthesis and sinusoidal efflux of GSH are undiminished or increased, so that the decrease in plasma GSH is indeed due to increased degradation of circulating GSH (Cho et al., 1981, 1984; Lauterburg et al., 1984a).

3. LOCALIZATION

3.1. TISSUE DISTRIBUTION

As mentioned earlier, glutathione is present in all mammalian cells. However, given the importance of its role in detoxifying exogenous toxins and sources of oxygen stress it may be particularly critical in cells in direct contact with the outside world, such as the lung and intestine.

Depletion of intestinal GSH with BSO \textit{in vivo} causes severe degeneration of enterocytes. Depletion of GSH levels in the intestinal epithelium can be partially overcome and toxicity can be prevented by oral administration of GSH or GSH monoester (Mårtensson et al., 1990). This suggests that GSH is needed for the preservation of normal intestinal function. Glutathione present in the diet and perhaps also GSH excreted into the bile may be sources of GSH in the enterocyte and may therefore be of great importance.

The lung is exposed to oxygen, oxygen radicals produced by alveolar macrophages, environmental toxins which are inhaled and, due to its position within the circulation, also to all toxins present in the blood. The first line of defense against oxidant stress and inhaled toxins may be extracellular GSH present in the epithelial lining fluid (ELF) of the lower respiratory tract, which is present in high concentrations. In healthy volunteers the average concentration of glutathione in the ELF is extremely high (430 \( \mu \text{M} \)) and predominantly in the reduced form. Toxic oxygen radicals may be reduced extracellularly by glutathione, since glutathione peroxidase, NADP and GSSG reductase are also present in the ELF (Cantin et al., 1987). Glutathione concentrations in the ELF are only 25% of normal values in idiopathic pulmonary fibrosis (Cantin et al., 1989), suggesting that oxidant stress due to GSH deficiency might be a contributing factor in the pathogenesis of this disease.

In contrast to inhaled toxins, circulating toxins are more likely to first encounter intracellular GSH. Depletion of intracellular GSH in the mouse causes morphological damage to the alveolar type II cells and the pulmonary endothelial cells, which is reversed by administration of GSH monoester (Mårtensson et al., 1989). In isolated rat alveolar type II cells exogenous, intact GSH taken up by the cells protects against paraquat (Hagen et al., 1986). Uptake of intact GSH has also been shown in the isolated rat lung model (Berggren et al., 1984; Dawson et al., 1984), but studies in the isolated rat lung and \textit{in vivo} studies in the mouse suggest that plasma GSH is predominantly metabolized to its precursors extracellularly and resynthesized (Berggren et al., 1984; Mårtensson et al., 1989). Thus, either by uptake of the intact molecule or by degradation and resynthesis, plasma GSH may be utilized by the lung for intracellular defense against circulating toxins. There is now evidence to suggest that uptake by the lung may be the major route of clearance of plasma GSH that escapes the kidney (Berggren et al., 1984; Mårtensson et al., 1989).

The renal requirement for detoxification of toxins by GSH is particularly high, since the kidney is exposed to concentrated solutions of toxins. The rapid turnover of GSH in the kidney may be very significant in this regard. However, variation in steady-state intracellular GSH levels in different parts of the kidney may also determine the localization of injury by toxins. In the rabbit GSH levels are lowest in the renal inner medulla and highest in the outer medulla and cortex (Mohandas et al., 1984). This may account for the selective damage to the inner medulla seen with chronic acetaminophen usage.

There also appears to be zonation of intracellular GSH concentration within the hepatic acinus. Periportal hepatocytes have GSH levels that are approximately twice the levels present in perivenular hepatocytes (Smith et al., 1979). Induction of GSH by phenobarbital does not change this concentration gradient (Väänänen, 1986). Isolated hepatocytes can be selectively obtained from zone 1 and zone 3, using the digitonin/collagenase perfusion method, and used to study GSH turnover. Using hepatocytes obtained by this method, GSH synthetic capacity has been found to be lower in zone 3 than in zone 1 hepatocytes (Kera et al., 1988). This may be due to differences in the activity of \( \gamma \)-glutamylcysteine-synthetase, the rate-limiting enzyme of GSH formation, which has been reported to be lower in isolated zone 3 hepatocytes (Penttilä, 1990).
Furthermore, there seems to be diminished availability of cysteine as a precursor for GSH. This appears to be due to a relative shift of cysteine towards taurine synthesis rather than GSH synthesis in zone 3 as compared to zone 1 hepatocytes (Penttilä, 1990). Conversely, cytochrome P-450 activity is higher in zone 3, so that enhanced activation of potential toxins occurs in the zone with the least GSH available to detoxify toxic metabolites. This may account for the centrilobular pattern of injury seen with several toxic compounds.

Glutathione is present in the astroglia and the endothelium of the blood–brain barrier in millimolar concentrations (Sivkova et al., 1987; Raps et al., 1989). It is unclear what role GSH plays in the brain, but it may be important in anti-oxidant defense. Brain capillaries demonstrate high γ-glutamyltranspeptidase activity on the luminal membrane, allowing extracellular hydrolysis and uptake of the constituent amino acids (Orlowski et al., 1974). Furthermore, there is evidence to suggest that GSH is taken up intact by carrier-mediated transport across the blood–brain barrier with an efficiency comparable to that of cysteine (Kannan et al., 1990).

### 3.2. Mitochondrial Glutathione

Between 10 and 15% of total intracellular GSH is located in the mitochondria, with 85 to 90% present in the cytosol (Wahländer et al., 1979; Meredith and Reed, 1982). Since the mitochondria do not have the enzymes necessary for GSH synthesis, the mitochondria import cytosolic GSH (Griffith and Meister, 1985).

Because of the central role the mitochondrion plays in the respiration of the cell, it is under physiological oxidant stress. It has been estimated that 3–5% of the oxygen consumed by mitochondria leads to toxic oxygen radicals. Since catalase is compartmentalized in the peroxisome, mitochondrial GSH in the presence of GSH peroxidase is the only defense against toxic oxygen intermediates. The anti-oxidant function of mitochondrial GSH is therefore critical for the viability of aerobic cells in the face of physiological and pathological oxidant stress.

Since the mitochondrion is unable to export GSSG (Olafsdottir and Reed, 1988), this organelle may be more susceptible than the rest of the cell to the consequences of a shift in the redox state. This might explain why mitochondrial GSH levels appear to be more closely linked to cell damage than cytosolic GSH levels do.

Glutathione in the mitochondrion appears to play a key role in maintaining calcium homeostasis. Mitochondria can take up and retain calcium, thereby contributing to the regulation of cytoplasmic calcium levels. Under pathological conditions, oxidation or depletion of mitochondrial GSH and oxidation of pyridine nucleotides appears to increase the permeability of the inner membrane of the mitochondrion to calcium. This may lead to mitochondrial calcium release, increased cytosolic calcium and, especially when other calcium-regulating pathways are disrupted, cell death (Lehninger et al., 1978; Lötcher et al., 1980; Moore et al., 1985).

Several experimental models have demonstrated that depletion of mitochondrial GSH can be a key step in the chain of events leading to cell death. In the isolated rat hepatocyte a concentration of 1,3-bis(2-chloroethyl)-l-nitrosourea (BCNU) was established that induced complete depletion of cytosolic GSH with partial preservation of mitochondrial GSH levels. An adriamycin concentration that caused modest depletion of both cytosolic and mitochondrial levels of GSH was also determined. At these concentrations adriamycin was not toxic and BCNU caused only modest increases in LDH leakage from hepatocytes. However, exposing hepatocytes to the combination of these two drugs did deplete mitochondrial GSH and caused a marked increase in LDH leakage. These findings suggest that the toxicity caused by the combination of these two compounds is due to the severe depletion of mitochondrial glutathione (Meredith and Reed, 1983).

The importance of mitochondrial GSH is also suggested by the finding that the toxicity of ethacrynic acid in hepatocytes is not manifest when cytosolic GSH is severely depleted, but rather at a later point in time when mitochondrial GSH is also greatly diminished (Meredith and Reed, 1982).

In the intact mouse, high doses of 3'-hydroxyacetanilide are not toxic, whereas the regiosomer 4'-hydroxyacetanilide, known as acetyaminophen, is toxic. Both compounds are able to deplete cytosolic GSH, but the toxic isomer, acetyaminophen, causes much more significant reductions in mitochondrial GSH than the non-toxic 3'-hydroxyacetanilide (Tirmenstein and Nelson, 1989). This depletion of mitochondrial GSH by acetyaminophen is accompanied by disruption of the ability of mitochondria to sequester calcium (Moore et al., 1985; Tirmenstein and Nelson, 1989). This raises the possibility that a component of acetyaminophen hepatotoxicity manifested as a delayed oxidant stress in mitochondria (Jaeschke, 1990) is directly related to mitochondrial GSH depletion rather than covalent binding of reactive metabolites to protein thiols.

After exposure of the isolated rat hepatocyte to the calcium ionophore, A23187, there is first a drop in cytosolic GSH, followed by a decline in mitochondrial GSH. Cell injury is not noted until after the fall in mitochondrial GSH. Furthermore, the leakage of LDH from the dead cells correlates best with mitochondrial GSH levels (Olafsdottir et al., 1988).

In chronic ethanol-fed rats there is a selective decrease in mitochondrial GSH pool size at all cytosolic GSH values. This is due to an impairment of transport of cytosolic GSH into the mitochondria.
mitochondrial GSH uptake may contribute to oxidant stress. It has been shown that oxidant stress induced by tertbutylhydroperoxide is more toxic to hepatocytes from ethanol-fed rats than to normal hepatocytes. Normalization of mitochondrial GSH with glutathione monoethyl ester will protect against this increase in susceptibility under conditions where cytosolic GSH is still depleted (Fernández-Checa et al., 1991a).

Severe depletion of GSH in skeletal muscle leads to damage which is associated with mitochondrial damage noted on electron microscopy. Restoration of mitochondrial GSH with GSH monoethyl ester prevents the skeletal muscle damage (Mårtensson and Meister, 1989). Mitochondrial GSH levels appear to be more closely linked to cell damage than cytosolic GSH levels do.

These examples of the correlation between toxicity and mitochondrial GSH have increased our appreciation of the pivotal role of mitochondrial GSH in sustaining cell viability and the potential significance of glutathione depletion in the pathophysiology of toxicity.

4. GLUTATHIONE TURNOVER

4.1. GLUTATHIONE SYNTHESIS

Glutathione synthesis from its three amino acid precursors, L-glutamate, L-cysteine, and glycine, takes place in the cytosol. It is a two step reaction, catalyzed by γ-glutamylcysteine synthetase and by GSH synthetase, that requires 2 moles of ATP per mole of GSH (Fig. 4).

γ-Glutamylcysteine synthetase catalyses the rate-limiting first step in which an amide linkage is formed between cysteine and the γ-carboxyl of glutamate:

\[
\text{L-glu + L-cys + ATP} \rightarrow \gamma\text{-glutamylcysteine} + \text{ADP.}
\]

GSH synthetase catalyses the second step in which glycine is linked to the cysteine carboxyl of γ-glutamylcysteine:

\[
\gamma\text{-glutamylcysteine + gly + ATP} \rightarrow \text{GSH + ADP.}
\]

Since ATP is required for GSH synthesis, GSH may be depleted when ATP levels are diminished. Since the \( K_m \) for these enzymes is very low, this should require profound depletion of ATP. Thus severe hypoxia and ischemia might be expected to impair anti-oxidant defense. This has been confirmed in hypoxic and anoxic hepatocytes which are more susceptible to the toxicity of peroxides (Tribble et al., 1988). However, the low \( K_m \) for ATP suggests that GSH synthesis is relatively protected from hypoxia.

The first step, catalyzed by γ-glutamylcysteine synthetase, is the rate-limiting enzyme and is controlled by negative feedback from its end product, GSH, by non-allosteric competitive inhibition (Richman and Meister, 1975). When GSH is consumed and feedback inhibition is lost, availability of cysteine as a precursor can become the limiting factor. To the limited degree in which cysteine is available to cells it can be taken up by via the ASC system. However since cysteine rapidly auto-oxidizes to cystine, plasma concentrations are low (5–10 \( \mu M \)) compared to cysteine levels (100–200 \( \mu M \)). Alternate sources of cysteine are the uptake of cystine with subsequent intracellular reduction and cysteine production from methionine by the cystathionine pathway. Freshly isolated hepatocytes and isolated perfused liver cannot utilize cystine, but the \( X_c \) system by which cystine can be taken up, is present in cultured hepatocytes (Takada and Bannai, 1984). Erythrocytes, lymphocytes and brain endothelium cannot utilize cystine and are therefore dependent on plasma cysteine.

There are two pathways by which plasma cystine can be converted to cysteine: (1) cells which can transport cystine may reduce it intracellularly and release cysteine into plasma; (2) GSH released from hepatocytes can liberate a cysteine from cystine by thiol–disulfide exchange according to the reaction:

\[
\text{GSH + Cys} \rightarrow \text{GS-Cys + CysH.}
\]

It has not been established to what degree these pathways contribute to plasma cysteine levels physiologically. The transsulfuration pathway in the liver (Fig. 5), which forms cysteine from methionine and serine at the expense of one additional ATP, is very active (Binkley and du Vigneaud, 1942; Binkley, 1951). Interestingly, both in isolated hepatocytes and in the perfused liver methionine has been shown to inhibit GSH efflux by a competitive type allosteric effect exerted at the external surface of the hepatocyte (Aw et al., 1984, 1986; Fernández-Checa et al., 1990). Thus methionine may have a dual role in sustaining hepatic GSH both by serving as a precursor for cysteine via the transsulfuration pathway and by inhibiting GSH efflux.

In contrast to the liver, the kidney does utilize cystine as a source of cysteine (Ormstad et al., 1980), but the low cystathionase activity does not permit active transsulfuration of methionine (Moldén et al.,...
4.2. EFFLUX FROM THE LIVER

The first step in the degradation of glutathione synthesized in the liver is efflux across either the sinusoidal or canalicular membrane (Fig. 6). The characteristics of the transport across the sinusoidal and canalicular poles is quite different and will be discussed separately.

4.2.1. Sinusoidal Efflux

Studies in the perfused liver have shown that virtually all the glutathione exported across the sinusoidal membrane is in the form of GSH (Bartoli and Sies, 1978; Hill and Burk, 1982; Kaplowitz et al., 1983; Ookhtens et al., 1985). Evidence for carrier-mediated transport of sinusoidal GSH has been provided by 3 different models. Sinusoidal transport of GSH has been shown to be saturable in hepatocytes isolated from animals at an age when 90% of efflux is sinusoidal (Ookhtens and Maddatu, 1991; Aw et al., 1986). Saturability has also been demonstrated in the perfused liver model (Ookhtens et al., 1985) and in studies with sinusoidal membrane vesicles (Inoue et al., 1984; Aw et al., 1987). A second characteristic of carrier-mediated process, trans-stimulation, has also been suggested. Preloading of
vesicles with GSH stimulated the uptake of GSH (Aw et al., 1987). Finally, competitive inhibition also is suggestive of carrier-mediated transport. As mentioned in an earlier section, methionine present at the outside of the cell inhibits GSH efflux from both isolated hepatocytes (Aw et al., 1984, 1986) and in the perfused rat liver model (Kaplowitz et al., 1989). Organic anions such as unconjugated and conjugated bilirubin, BSP-GSH, BSP, rose bengal and indocyanine green are all inhibitors of sinusoidal GSH efflux in the perfused rat liver model (Ookhtens et al., 1988). In the isolated cell model unconjugated and conjugated bilirubin were found to be competitive inhibitors of GSH efflux (Ookhtens et al., 1988). Inhibition was found to occur from within the cell, suggesting competition for carrier sites.

Several other factors have been found that influence sinusoidal efflux rates. There is a decrease in efflux in the perfused rat liver with increased age of the animals (Ookhtens et al., 1987). Membrane potential may also be an important determinant of GSH transport, possibly because it has a net negative charge at physiological pH. Studies in membrane vesicles suggest that an inwardly directed K⁺ gradient stimulates electrogenic, potential driven transport of GSH (Aw et al., 1987).

Hormonal regulation of GSH efflux has been another area of some interest. It has been shown that hormones and vasoactive substances, such as vasopressin, phenylephrine and norepinephrine, increase sinusoidal efflux by calcium- and C-kinase-dependent signal transduction (Sies and Graf, 1985). However other investigators have found that calcium and C-kinase increase permeability of hepatocyte tight junctions (Ballatori and Truong, 1989). This might suggest that the apparent increase in sinusoidal GSH is due to leakage of canalicular GSH through the more permeable tight junctions. The lack of effect of vasopressin and phenylephrine on GSH efflux in isolated hepatocytes is consistent with the latter explanation (Lu et al., 1990). The role of cAMP in stimulating sinusoidal GSH efflux is also controversial. Two studies have not found significant increases with glucagon (Sies and Graf, 1985; Wright et al., 1988), whereas other studies in the isolated hepatocyte and the perfused rat liver model demonstrated stimulation of efflux with glucagon and other stimulators of cAMP (Lu et al., 1990).

Pathological conditions may also alter sinusoidal export of GSH. Chronic exposure to alcohol enhances efflux in both isolated hepatocytes and in the perfused rat liver model (Pierson and Mitchell, 1986; Fernández-Checa et al., 1987). This effect appears to be due to increased affinity for transport of hepatic GSH by an unknown mechanism.

4.2.2. Canalicular Efflux

About three-quarters of the glutathione excreted across the canalicular membrane is in the reduced form (Eberle et al., 1981; Ballatori et al., 1989). A substantial amount of the GSH excreted into the bile is hydrolyzed by γ-glutamyltransferase and dipeptidase on the external surface of the biliary ductular epithelium to cyst(e)inylglycine, γ-glutamylglutathione, γ-glutamylcyst(e)ine, cyst(e)ine, glutamate and glycine (Abbott and Meister, 1986; Ballatori et al., 1986). There are transport systems for both glycine and glutamate present, but the physiological contribution of intrahepatic recirculation of these amino acids remains to be established (Ballatori et al., 1988; Moseley et al., 1988).

Studies in the isolated perfused rat liver in which γ-glutamyltransferase has been inhibited with acivicin suggest that biliary GSH efflux accounts for up to 50% of total hepatic efflux (Ballatori et al., 1989). The relative contribution of sinusoidal and canalicular excretion does vary with age of the animal and hepatic concentration. In the young rat (25–45 days old or 60–240g) 5% of total efflux is biliary, whereas in the adult rat (110–170 days old or 400–555 g) this percentage increases to 60% (Ookhtens and Maddatu, 1991). Since the rat does not reach maturity until 6 months, this is a developmental change rather than a manifestation of aging. Canalicular efflux of GSH is also concentration dependent, without evidence of capacity-limitation, so that efflux is dependent on hepatic concentration (Kaplowitz et al., 1983; Ookhtens and Maddatu, 1991).

Although non-saturable transport is not usually seen in a carrier-mediated process, there is some evidence suggestive of a carrier-mediated process. Inhibition of canalicular efflux by organic anions such as bromosulphthalein (Ballatori and Clarkson, 1985) and induction of efflux by phenobarbital is consistent; with carrier-mediated transport (Kaplowitz et al., 1983). Furthermore, in isolated canalicular membranes saturability could be demonstrated (Inoue et al., 1983). However, the physiological relevance of the saturable transport process in the isolated membranes is unclear, since the affinity constant (Kᵦ = 0.33 mm) was low in comparison to normal (5–10 mm) hepatic GSH concentration. Therefore this transport pathway would be saturated even under conditions in which glutathione was severely depleted. A final piece of evidence suggesting that canalicular efflux is a carrier-mediated process comes from studies done in rats with defective organic anion excretion. In mutant rats with this defect known as TR⁻, the hereditary conjugated hyperbilirubinemia and the defect in biliary organic anion is accompanied by normal to high hepatic GSH levels. Yet despite the normal to high intracellular levels of GSH there is only trace biliary GSH efflux in intact TR⁻ rats and in the isolated perfused liver model, which would not be consistent with passive diffusion (Oude Elferink et al., 1989). However, another explanation for the absence of GSH in the bile of these mutant rats is that there is accumulation of an inhibitor of GSH transport due
to the defective organic anion transport. On the other hand, recent studies provide evidence against active transport. Canaliculal membrane vesicles from normal rats and from EHBR mutant rats, which seem to have the same defect as the TR− rats, do not show ATP-dependent stimulation of GSH transport, suggesting that canaliculal efflux is not a primary active transport process (Fernández-Checa et al., 1991b).

Transport across the sinusoidal and canaliculal membranes appears to occur by different mechanisms, so that it is not surprising that the two poles respond differently to various factors. With increasing age canaliculal efflux increases (Abbott and Meister, 1986; Ookhtens and Maddatu, 1991; Ballatori and Clarkson, 1982; Gregus et al., 1987) at the expense of sinusoidal efflux (Ookhtens et al., 1987). In the perfused rat liver model exposure to phenolic anti-oxidants also increases canaliculal efflux with a concomitant decrease in sinusoidal efflux (Jaeschke and Wendel, 1988). Thus, some factors cause reciprocal changes at the sinusoidal and canaliculal membranes. On the other hand, studies in isolated membrane preparations show that bromo-sulphalein, an organic anion, inhibits both sinusoidal and canaliculal efflux (Ballatori and Clarkson, 1985; Inoue et al., 1983). However, BSP-GSH, which is also an organic anion, selectively inhibits sinusoidal efflux.

Canaliculal GSSG efflux: biliary GSSG release increases with oxidative stress in the cytosol (Akerboom et al., 1982; Lauterburg et al., 1984b). This presumably occurs when detoxification of hydroperoxides leads to generation of GSSG in excess of that which can be reduced back to GSH by the GSSG reductase system. In isolated rat liver canaliculal membranes GSSG transport is saturable with a Ka of 1.0 mM in the absence of ATP, suggesting a carrier-mediated process (Inoue et al., 1984). However, there appears to be an ATP-dependent process. In the perfused rat liver the GSSG gradient between bile and hepatocyte cytosol is 50-fold, consistent with active transport (Akerboom et al., 1982). Other evidence which supports the requirement for ATP is the fall in biliary GSSG efflux after exposure to intracellular mitochondrial uncouplers (Sies, 1983). Furthermore, GSSG dependent ATPase activity has been reported in hepatic plasma membranes. Finally, ATP dependent GSSG transport has been demonstrated in canaliculal liver plasma membrane vesicles. It is absent in mutant TR− and EHBR rats suggesting its transport is shared with organic anions. Canaliculal transport of GSSG seems to be shared by GSH-conjugates but not GSH.

Glutathione disulfide transport in the erythrocyte is also an ATP-requiring process (Srivastava and Beutler, 1969c), mediated by two Mg2+-ATPases (Kondo et al., 1987, 1989). The characteristics of the transporter have been studied in inside-out vesicles from human red cells (Kondo et al., 1980, 1982). In this model a high and a low affinity component have been identified with an apparent Ka of 0.1 and 7 mM respectively (Kondo et al., 1980). Glutathione conjugates are also exported by ATP-dependent transport in the erythrocyte (Kondo et al., 1982; Board, 1981; LaBelle et al., 1986), but it has not been established whether these conjugates share a transporter with GSSG (Kondo et al., 1980; LaBelle et al., 1986; Eckert and Eyer, 1986).

GSSG transport in the heart is a saturable, ATP-requiring process as well (Xia et al., 1985; Ishikawa et al., 1986). Analogous to the canaliculal membrane and the erythrocyte, there appears to be a common pathway for GSSG and the glutathione conjugates (Ishikawa and Sies, 1984).

### 4.3 INTERORGAN HOMEOSTASIS

There is constant turnover of glutathione in the body. Synthesis of GSH occurs predominantly in the liver, with subsequent release of GSH across the sinusoidal membrane into plasma and breakdown to the component amino acids mainly in the kidney and lungs.

One of the main sources of cysteine for GSH synthesis by the liver is the trans-sulfuration pathway which allows conversion of methionine and serine to cysteine. Hepatic GSH exported across the sinusoidal membrane accounts for ninety percent of GSH present in the plasma (Lauterburg et al., 1984a). Clinically, the relationship between plasma GSH and normal hepatic synthesis and efflux is demonstrated in cirrhosis. In cirrhosis plasma GSH is diminished due to decreased GSH input into the circulation, which most likely reflects decreased liver efflux (Burgunder and Lauterburg, 1987). Since the rat liver does not have very much sinusoidal γ-glutamyltranspeptidase, it cannot take up GSH from the plasma. In contrast, both guinea pig and human liver may have significant activity of γ-glutamyltranspeptidase on the plasma membrane.

Until recently the kidney was believed to account for 50–70% of clearance from plasma (Hahn et al., 1978; Griffith and Meister, 1979; Häberle et al., 1979). However, newer evidence suggests that the lung also clears a substantial amount of the plasma GSH through hydrolysis by γ-glutamyltranspeptidase and uptake into the lung (Berggren et al., 1984; Mårtensson et al., 1989). First pass extraction of GSH through the kidney may be as high as 80–90% (Bartoli et al., 1978). If clearance was strictly by glomerular filtration only 25–30% could be cleared on a first pass, so that the remainder must be removed by tubular extraction. γ-glutamyltranspeptidase and dipeptidase are present on the external surface of the luminal and basolateral membranes of the proximal tubule. Glutathione that is filtered by the glomerulus will be hydrolyzed by the enzymes on the luminal
membrane (Abbott et al., 1984). The three constituent amino acids are then utilized to resynthesize glutathione, either by the epithelium of the proximal tubule or, after circulating in the plasma, by the liver and other organs. Uptake of glutathione from the peritubular plasma occurs both through the hydrolytic pathway described above as well as through uptake of the intact tripeptide (Lash and Jones, 1984).

Glutathione efflux from the liver does increase in response to greater utilization by peripheral organs. For example, skeletal muscles require more glutathione to cope with the oxidative stress induced by exercise. In rats exercised to exhaustion, the increased need for glutathione in skeletal muscle leads to a drop in muscle and liver GSH and an increase in plasma levels (Lew et al., 1985; Pyke et al., 1986). Similarly, hepatic efflux increases with fasting (Lauterburg et al., 1984a), possibly to provide cysteine for protein synthesis in peripheral tissues. This is probably an area in which the hormonal regulation of hepatic glutathione plays a key role.

Depending on the age of the animal and the level of hepatic GSH, up to half of all GSH excreted by the liver is transported across the canalicular membrane (Ballatori et al., 1989; Ookhtens and Maddatu, 1991). The metabolism and function of GSH in the bile is probably quite different from that present in the bloodstream. There is significant breakdown of GSH within the biliary tract and the amino acids released might be subject to intrahepatic recirculation (cholehepatie shunting). Another function of biliary GSH may be as a mucolytic agent that prevents stone formation in the biliary tree. Glutathione excreted by this route might also be taken up by the intestine. From the available evidence it is not clear whether glutathione present in the intestinal lumen would be taken up intact or would be degraded extracellularly by \( \gamma \)-glutamyltranspeptidase. The brush border of intestinal villus tip cells is rich in \( \gamma \)-glutamyltranspeptidase (Cornell and Meister, 1976). On the other hand, several studies have provided evidence for carrier-mediated uptake of intact GSH in the enterocyte (Lash et al., 1986; Hagen and Jones, 1987; Vincenzini et al., 1988), although the relative importance of Na\(^+\)-dependent (Hagen and Jones, 1987; Vincenzini et al., 1988; Linder et al., 1984) and Na\(^+\)-independent pathways remains uncertain (Vincenzini et al., 1988; Linder et al., 1984; Hunjan and Evered, 1985). Furthermore, uptake of exogenous intact GSH has been found to protect the intestinal epithelial cells from oxidant injury (Lash et al., 1986). If GSH is taken up intact, oral administration of large doses might be used therapeutically to increase plasma and tissue GSH. *In vivo* studies in the rat suggest that this might indeed be feasible (Hagen and Jones, 1987).

### Table 1. Glutathione S-Transferase Gene Superfamily

<table>
<thead>
<tr>
<th>Family</th>
<th>Human</th>
<th>Rat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>GST2</td>
<td>1-1</td>
</tr>
<tr>
<td></td>
<td>B(_1) (_1)</td>
<td>2-2</td>
</tr>
<tr>
<td></td>
<td>B(_2) (_2)</td>
<td>8-8</td>
</tr>
<tr>
<td>Mu</td>
<td>GST1</td>
<td>3-3</td>
</tr>
<tr>
<td></td>
<td>GST4 (muscle)</td>
<td>3-6</td>
</tr>
<tr>
<td></td>
<td>GST5 (brain)</td>
<td>4-6</td>
</tr>
<tr>
<td>Pi</td>
<td>GST3</td>
<td>7-7</td>
</tr>
</tbody>
</table>

Distribution in humans and rats of the glutathione S-transferases according to the classification by superfamilies.

*Transferase 5–5 is not yet classified by family.

### 5. GLUTATHIONE S-TRANSFERASES

#### 5.1. NOMENCLATURE

There are multiple glutathione S-transferases (GST) and several methods have been developed over the years to classify the different families (see reviews for details: Ketterer, 1986; Mannervik and Danielson, 1988; Boyer, 1989). The first classification was based on substrate specificity (Boylan and Chasseaud, 1969), whereas subsequent systems attempted to use differences in chemical separation characteristics (see Table 1). Separation based on substrate specificity was complicated by the extensive overlap in substrates catalyzed by different isozymes. In the classification based on elution from carboxymethyl cellulose ion exchange in the rat, GST isozymes are called AA, A, B, C, D, and E (Jakoby, 1978). Subunits are labelled Y\(_a\), Y\(_b\) and Y\(_c\) in the classification system based on the relative mobilities of the subunits on SDS-PAGE (Bass et al., 1977). Since each isozyme is composed of 2 subunits, this system easily distinguishes the different combinations of homodimers and heterodimers. The most recent classification of rat enzymes based on chemical separation techniques, was developed in response to the increasing number of GSTs identified. In this classification Arabic numerals designate each subunit (Jakoby et al., 1984). In all of these systems the species must be specified, since overlap between species has not been well studied.

The most recent classification is based on extensive interspecies structural and functional homology noted within three classes or 'families': GST \( \alpha \), \( \mu \) and \( \pi \) (Mannervik et al., 1985). GST \( \alpha \) class has a basic isoelectric point, has GSH peroxidase activity and functions as ligandin. GST \( \mu \) has a near neutral...
isoelectric point and is the main transferase that conjugates and thereby detoxifies epoxides. Three autosomal alleles for GST μ have been described in humans: Type 1, type 2 and the null type, in which there is an absence of GST μ activity. Activity of GST μ can be measured in circulating monocytes (Seidégård and Pero, 1988) and this has allowed population studies of the prevalence of the alleles. In a European population forty percent of individuals lack GST μ activity, i.e. have the null type (Strange et al., 1984). Since GST μ is responsible for the detoxification of many reactive metabolites that are potential carcinogens by catalyzing the conjugation to GSH, the null phenotype may be a risk factor for cancer. A study of the risk of developing lung cancer among heavy smokers was consistent with this hypothesis. Fifty-nine percent of heavy smokers who had not developed lung cancer expressed GST μ activity, whereas in those who had developed lung cancer only 30% expressed GST μ activity (Seidégård et al., 1986).

The GST π class of transferases have an acidic isoelectric point and are expressed in placenta, small intestine and bile ducts. The presence of this class of transferase in cancer cells may contribute to resistance to chemotherapy (Kithihara et al., 1984; Suzuki and Board 1984) and chromo-

Localization and expression of GST have been studied with molecular biological techniques. The three class α genes described to humans to date are located on chromosome 6 (Board and Webb, 1987; Islam et al., 1989). Two of these are expressed in the liver and one is expressed in the skin. The human class π genes are located on chromosome 1 and 3 (Islam et al., 1989; DeJong et al., 1988a), whereas human class μ is located on chromosome 11 (Laisney et al., 1983; Suzuki and Board 1984) and chromosome 12 (Board et al., 1989).

5.2. MECHANISM OF ACTION

Glutathione S-transferases are usually composed of 2 subunits, with a complete active site on each subunit (Danielson and Mannervik, 1985; Tahir and Mannervik, 1986). The active site has a highly specific binding site for glutathione adjacent to a partly hydrophobic binding site for the electrophilic substrate. γ-Glutamylcysteine, the immediate precursor in the GSH synthetic pathway, is the only other endogenous thiol substrate which will bind to the glutathione site, albeit much less avidly than GSH (Sugimoto et al., 1985).

Current thinking is that a structural basic amino acid in the glutathione binding site of the transferase promotes deprotonation of the thiol group of GSH, causing the thiol to ionize to a reactive thiolate ion (Graminski et al., 1989). The substrate may also be activated by a structural amino acid in the hydrophobic binding site that protonates the substrate and increases electrophilicity (Mannervik and Danielson, 1988).

5.3. FUNCTION

The major functions of the GST are: (1) catalysis of GSH conjugation of electrophiles; (2) peroxidation of lipid hydroperoxides; (3) intracellular transport (ligandin function); and (4) direct inactivation of electrophilic metabolites.

The conjugation of glutathione to electrophiles can be either spontaneous or catalyzed by GST. The necessity for enzymatic catalysis depends on the energy barrier that has to be overcome to cause the electrophile and nucleophile to react with each other. This will depend on the density of the charge of the two substrates. Compounds with centers of high charge density are called hard electrophiles or nucleophiles, whereas those with low charge density are called soft. The potential energy barrier to a reaction between two chemicals is lowest when chemicals of similar hardness or softness are involved. Glutathione is a soft nucleophile and will therefore react more rapidly with soft electrophiles (Coles, 1985). Enzymatic catalysis by GST will therefore more likely be needed for conjugation of glutathione to hard electrophiles, whereas conjugation to soft electrophiles is more likely to occur spontaneously. However, GST may catalyze reactions with soft electrophiles such as NAPQI and catalyzed conjugation predominates at low GSH concentrations (Coles et al., 1988) when nearly all the GSH is bound to GST (Sugiyama and Kaplowitz, 1984). The other determinant of a reaction rate, and therefore for the potential enhancement by an enzyme, is the concentration of the substrates. Catalysis by GST assumes greater importance under conditions of GSH depletion or when the electrophile is present in low concentrations.

The second function of the GST is intracellular transport. A class of intracellular carrier proteins in the liver was originally designated ligandin. Subsequently, ligandin and GSH-S-aryltransferase were found to be quite similar (Kaplowitz et al., 1973) and ligandin was ultimately found to be identical.
Glutathione metabolism and hepatotoxicity

6. GLUTATHIONE THERAPY

Pharmacological interventions that decrease or increase intracellular glutathione may be of therapeutic value. In the field of cancer research, the former has been examined as a way to increase the efficacy of chemotherapy. Many cytotoxic drugs are detoxified by GSH, so that selectively decreasing intracellular GSH in tumors might enhance the therapeutic index. In vitro studies have shown that decreasing cellular GSH by depriving cells of GSH precursors or by adding buthionine sulfoximine, BSO (an inhibitor of γ-glutamylcysteine synthetase), restores sensitivity to 1-phenylalanine mustard (L-PAM) in resistant L1210 leukemic cells (Somfai-Relle et al., 1984; Suzukake et al., 1982). This has been confirmed in vivo: treatment of mice with BSO sensitizes L-PAM resistant L1210 leukemic cells inoculated intraperitoneally and leads to a small increase in lifespan (Somfai-Relle et al., 1984). The use of BSO as an adjuvant to chemotherapy in humans is currently under active investigation.

The use of precursors of GSH to bolster intracellular levels is also of potential therapeutic value. One well-established application is the use of N-acetylcysteine (NAC) as an antidote to acetaminophen toxicity (Lyons et al., 1977; Prescott et al., 1979; Rumack et al., 1981). NAC may be a direct source of cysteine following hydrolysis or may reduce plasma cysteine through thiol-disulfide exchange, liberating endogenous cysteine (Burgunder et al., 1989). In contrast to its ability to increase hepatic GSH under depleted conditions, under normal conditions NAC will not increase total glutathione, since the intracellular concentration is under feedback control.

L-2-oxothiazolidine-4-carboxylate is another compound that can support GSH synthesis by providing cysteine. This compound is converted to S-carboxy-L-cysteine by 5-oxo-L-prolinase and after spontaneous decarboxylation L-cysteine is liberated (Williamson and Meister, 1981). Experimental applications of oxothiazolidine have included oxygen toxicity (Tsan and Phillips, 1988) and acetaminophen toxicity in vivo in mice (Williamson et al., 1982).

The two compounds described above both support GSH synthesis under conditions of depleted levels by supplying a source of cysteine. An alternate method to boost GSH levels is by providing the cell directly with GSH. This has been proven possible by the administration of esters of GSH that are rapidly taken up by various cell types and hydrolyzed intracellularly (Puri and Meister, 1983). Unlike the cysteine prodrugs, this bypasses γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis, so that intracellular concentrations can be achieved which are greater than those found normally (Wellner et al., 1984). GSH monoester protects mice from acetaminophen toxicity (Puri and Meister, 1983). It has also been found to protect against the morphological damage seen in a model of chronic GSH depletion by BSO (Mårtensson et al., 1989). The therapeutic use of GSH monoesters such as the monoethylester may be limited by the amount of ethanol liberated intracellularly as the ester is hydrolyzed. If this problem can be circumvented with the use of other types of ester derivatives, this approach to augmenting intracellular GSH may have many therapeutic applications.

7. CONCLUSION

Glutathione is a key molecule in aerobic cells. The essential functions it fulfills include the detoxification of toxic oxygen and electrophilic metabolites, the preservation of essential thiol groups and the transport of cysteine. One of the most important developments in recent years in our understanding of the detoxification function has been the recognition of the importance of mitochondrial GSH, which may be the critical target of certain toxins.

The availability of GSH to various tissues is determined by the liver and kidney, which synthesize and release GSH and GSH precursors into the plasma. The liver is the only organ known to be able to derive cysteine from methionine, so that hepatic synthesis of GSH can be maintained in the face of cysteine deprivation. The main source of plasma GSH is hepatic GSH transported across the sinusoidal membrane and this process appears to...
respond to the needs of peripheral tissues. The kidney and lung are the main organs responsible for clearance of the tripeptide. The kidney releases intact GSH into the tubule, whereas the amino acid constituents derived from GSH are released into the blood, allowing utilization by other organs. Our knowledge of the factors that regulate intracellular turnover in the various organs and interorgan homeostasis is still limited, but the obvious complexity of the system seems in keeping with the importance of the functions it fulfills.

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