Glutathione: Systemic Protectant Against Oxidative and Free Radical Damage

Dedicated to the memory of Professor Daniel Mazia, my PhD mentor and a pioneer in cell biology

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Abstract

The tripeptide thiol glutathione (GSH) has facile electron-donating capacity, linked to its sulfhydryl (—SH) group. Glutathione is an important water-phase antioxidant and essential cofactor for antioxidant enzymes; it provides protection also for the mitochondria against endogenous oxygen radicals. Its high electron-donating capacity combined with its high intracellular concentration endows GSH with great reducing power, which is used to regulate a complex thiol-exchange system (—SH ⇌ —S-S—). This functions at all levels of cell activity, from the relatively simple (circulating cysteine/—SH thiols, ascorbate, other small molecules) to the most complex (cellular —SH proteins).

Glutathione is homeostatically controlled, both inside the cell and outside. Enzyme systems synthesize it, utilize it, and regenerate it as per the gamma-glutamyl cycle. Glutathione is most concentrated in the liver (10 mM), where the "P450 Phase II" enzymes require it to convert fat-soluble substances into water-soluble GSH conjugates, in order to facilitate their excretion. While providing GSH for their specific needs, the liver parenchymal cells export GSH to the outside, where it serves as systemic source of —SH/reducing power.

GSH depletion leads to cell death, and has been documented in many degenerative conditions. Mitochondrial GSH depletion may be the ultimate factor determining vulnerability to oxidant attack. Oral ascorbate helps conserve GSH; cysteine is not a safe oral supplement, and of all the oral GSH precursors probably the least flawed and most cost-effective is NAC (N-acetylcysteine).
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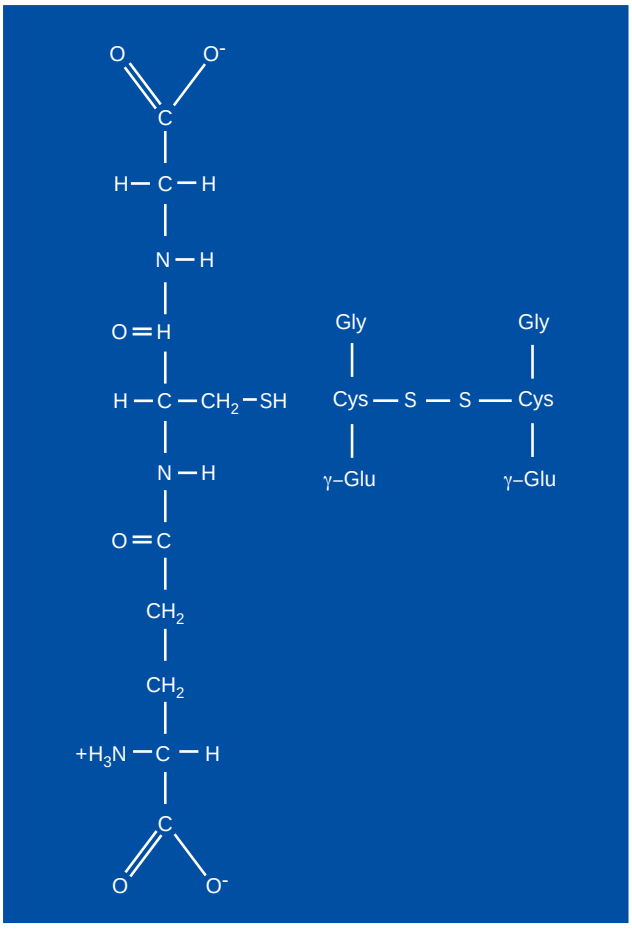
Glutathione (γ-glutamylcysteinylglycine, GSH) is a sulfhydryl (—SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is ubiquitous in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system.[1-4] Glutathione often attains millimolar levels inside cells, which makes it one of the most highly concentrated intracellular antioxidants.

Glutathione exists in two forms (Fig. 1): The antioxidant "reduced glutathione" tripeptide is conventionally called glutathione and abbreviated GSH; the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress.

Figure 1. Structure of GSH (reduced glutathione, left) and of GSSG (oxidized glutathione, right). From Stryer.[5]



GSH has potent electron-donating capacity, as indicated by the high negative redox potential of the GSH/GSSH "redox couple" (E'_0 =-0.33v).[6] Its high redox potential renders GSH both a potent antioxidant *per se* and a convenient cofactor for enzymatic reactions that require readily available electron pairs, the so-called "reducing equivalents."[7] Lewin[6] articulated how a substance with great readiness to donate electrons, when present at high concentrations, has greatly enhanced effectiveness as a reductant. This is reducing power, and is most expressed by GSH where its concentrations are highest (as in the liver). The reducing power of GSH is a measure of its free-radical scavenging, electron-donating, and sulfhydryl-donating capacity. Reducing power is also the key to the multiple actions of GSH at the molecular, cellular, and tissue levels, and to its effectiveness as a systemic antitoxin.[8]

The reduced glutathione molecule consists of three amino acids — glutamic acid, cysteine, and glycine — covalently joined end-to-end (Fig. 1). The sulfhydryl (—SH) group, which gives the molecule its electron-donating character, comes from the cysteine residue. Glutathione is present inside cells mainly in its reduced (electron-rich, antioxidant) GSH form. In the healthy cell GSSG, the oxidized (electron-poor) form, rarely exceeds 10 percent of total cell glutathione.[1] Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis.[9,10]

The peer-reviewed literature on glutathione is too extensive to be adequately discussed in a single review. This review summarizes the salient features of GSH as antioxidant and systemic protectant, examines instances of GSH abnormalities linked to tissue and organ system breakdown, and explores the possibilities for GSH replacement therapy to benefit degenerative conditions.

Glutathione Biosynthesis, Metabolism, and Utilization

The metabolism of GSH has been worked out to an extent that cannot be fully detailed herein; publications by the late Alton Meister and his colleagues provide greater detail.[11-14] Glutathione status is homeostatically controlled, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthetase enzymes), its recycling from GSSG (by GSH reductase), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases).

The overall picture of GSH metabolism is summarized by way of the gamma-glutamyl cycle in Fig. 2. Glutathione

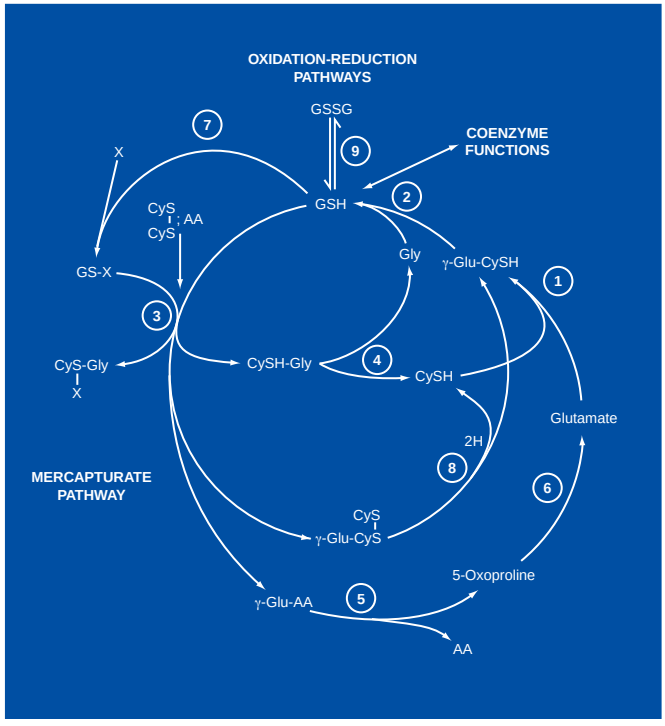
synthesis occurs within cells in two closely linked, enzymatically controlled reactions that utilize ATP and draw on nonessential amino acids as substrates. First, cysteine and glutamate are combined (by the enzyme gamma-glutamyl cysteinyl synthetase, see Reaction 1 in Fig. 2), with availability of cysteine usually being the rate-limiting factor. Cysteine is generated from the essential amino acid methionine, from the degradation of dietary protein, or from turnover of endogenous proteins. The buildup of GSH acts to feedback-inhibit this enzyme, thereby helping to ensure homeostatic control over GSH synthesis.

The second GSH synthesis reaction combines gamma-glutamylcysteine with glycine to generate GSH (catalyzed by GSH synthetase, Reaction 2 in Fig. 2). Excessive accumulation of gamma-glutamylcysteine in the absence of its conversion to GSH can lead to its conversion to 5-oxoproline by the enzyme gamma-glutamyl cyclotransferase (Reaction 4). Buildup of 5-oxoproline can have adverse consequences due to metabolic acidosis.

The GSH pool is drawn on for 3 major applications: (a) as cofactor for the GSG-S-transferases in the detoxicative pathways (Reaction 7 in Fig. 2); (b) as substrate for the gamma-glutamyl transpeptidases, enzymes which are located on the outer cell surface and which transfer the glutamine moiety from GSH to other amino acids for subsequent uptake into the cell (Reaction 3); and (c) for direct free-radical scavenging and as an antioxidant enzyme cofactor (Reaction 9). The GSH transferases are a large group of isozymes that conjugate GSH with fat-soluble substances as the major feature of liver detoxification. For further details of the gamma-glutamyl cycle, the reader is referred to Meister[11,12] and Anderson.[13]

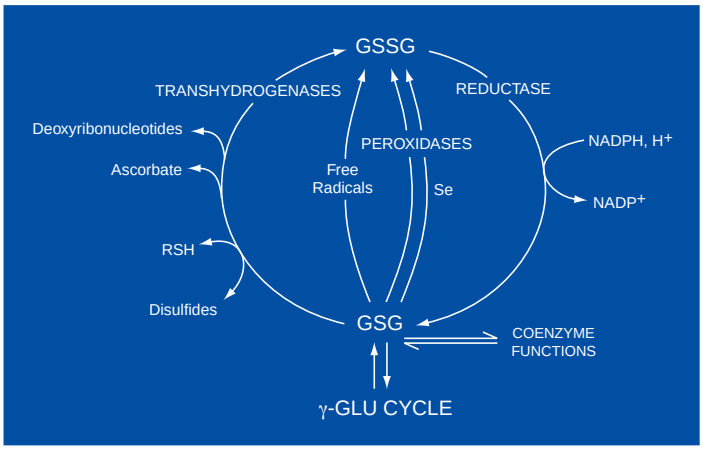
The oxidation-reduction pathways of GSH are summarized in Fig. 3. Glutathione is an essential cofactor for antioxidant enzymes, namely the GSH peroxidases (both Se-dependent and non-Se-dependent forms exist) and the more recently described phospholipid hydroperoxide GSH peroxidases.[15] The GSH peroxidases serve to detoxify peroxides (hydrogen peroxide, other peroxides) in the water-phase, by reacting them with GSH; the latter enzymes use GSH to detoxify peroxides generated in the cell membranes and other lipophilic cell phases.[16] This is one instance of the water-soluble GSH providing electrons to help reduce oxidized biomolecules located away from the water phase.

Figure 2. Scheme of overall GSH metabolism. From Meister.[12]



Enzymes collectively known as GSH transhydrogenases use GSH as a cofactor to reconvert dehydroascorbate to ascorbate, ribonucleotides to deoxyribonucleotides, and for a variety of —S-S— ⇌ —SH interconversions (Fig. 3). After GSH has been oxidized to GSSG, the recycling of GSSG to GSH

Figure 3. The oxidation-reduction pathways that involve glutathione. From Meister.[12]



is accomplished mainly by the enzyme glutathione reductase. This enzyme uses as its source of electrons the coenzyme NADPH (nicotinamide adenine dinucleotide phosphate, reduced). Therefore NADPH, coming mainly from the pentose phosphate shunt, is the predominant source of GSH reducing power. Cathcart used this to explain why subjects unable to make adequate NADPH may be at increased risk of oxidative damage from GSH insufficiency.[16]

Through its significant reducing power, GSH also makes major contributions to the recycling of other antioxidants that have become oxidized. This could be the basis by which GSH helps to conserve lipid-phase antioxidants such as alpha-tocopherol (vitamin E), and perhaps also the carotenoids. Meister and his group used buthionine sulfoximine (BSO) to inhibit GSH synthesis in rodents, and concluded from their findings that GSH almost certainly plays such a role *in vivo*.[8,12-14]

The liver seems to have two pools of GSH; one has a fast turnover (half-life of 2-4 hours), while the other is avidly retained with a half-life of about 30 hours.[14] The first corresponds to cytosolic GSH, the second mainly to mitochondrial GSH which is known to be more tightly held. Though this pool represents a minor portion of the total GSH, the mitochondria are normally under high oxidative stress[17] and thus conserve their GSH.

With regard to the essentiality of GSH for the survival of the whole organism, substantial information is available from studies on hereditary GSH depletion in the human, and from experimental depletion and repletion of GSH in animal models and cell cultures.[11,18]

Inherited deficiency of the enzyme gamma-glutamyl cysteine synthetase, the first of the two enzymes necessary for GSH synthesis, has been described in two human siblings. They exhibited generalized GSH deficiency, hemolytic anemia, spinocerebellar degeneration, peripheral neuropathy, myopathy, and aminoaciduria, and severe neurological complications as they moved into their fourth decade of life.[11] Their red cell GSH was less than 3% of normal, their muscle GSH less than 25%, and their white cell GSH less than 50% normal. One of them may have been hypersensitive to antibiotics, having developed psychosis after a single dose of sulfonamide for a urinary tract infection.

Deficiency in GSH synthetase, the second enzyme of GSH synthesis, also is associated with hemolytic tendency and defective central nervous system function. This condition is complicated by the metabolic consequences of an excess of 5-oxoproline, formed as a "spillover" from the accumulation of gamma-glutamylcysteine after its normal synthesis by the first enzyme and its lack of conversion to GSH by the second enzyme.[11,18]

Human hereditary GSH deficiency states are not necessarily lethal, probably because some GSH is obtained directly from the diet. With laboratory animals it is possible to precisely control GSH in the diet. Meister's group set dietary GSH at zero for their experimental animals, and simultaneously blocked endogenous GSH synthesis (at the first step, using buthionine sulfoximine).[13,14] They observed that GSH levels decreased in the plasma, liver, kidney, and other tissues of these animals; in guinea pigs and newborn rats death

ensued within a few days. At the cell level, the damage mostly involved the mitochondria, but nuclear changes were also observed. Lung Type 2 cells showed damage to their lamellar bodies, the vesicles that package lung surfactant and release it to the cell exterior. This damage from GSH depletion could be ameliorated by simultaneously administering precursors of GSH; thus the cataracts in newborn rats were blocked using orally-administered GSH monoesters.

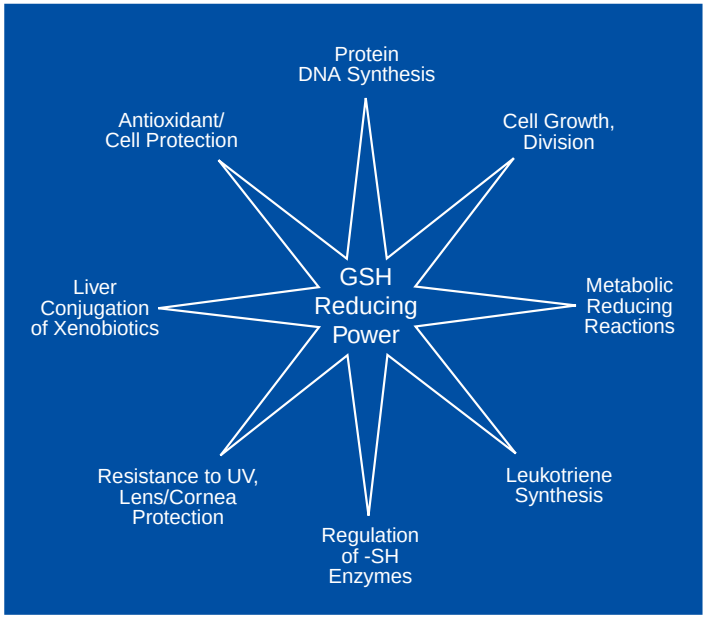
Meister, Anderson, and collaborators reasonably assumed that the damage produced in their test animals from inhibition of GSH synthesis was endogenous, since they had not applied any exogenous sources of oxidative challenge. The mitochondria appeared to be the most susceptible foci in the GSH-depleted tissues. This finding was consistent with the mitochondria assuming the bulk of the endogenous oxygen radical burden, yet, being unable to make their own GSH, they must import it from the cell cytosol.

The investigators found that dietary ascorbate can protect against the tissue damage that typically results from depletion of GSH.[13,14] In animals such as adult rats and mice who are able to make adequate ascorbate on their own, GSH depletion was not lethal. By contrast, in those animals that could not make their own ascorbate (newborn rats, guinea pigs), GSH depletion was lethal. Supplementation of the diet with ascorbate protected these animals against GSH depletion and saved their lives. Interestingly, this story has a "flip side"- guinea pigs placed on an ascorbate-deficient diet were salvaged by dietary administration of GSH and its precursors.[13,14] Thus, these two water-phase antioxidants are tightly linked: GSH can conserve ascorbate *in vivo*, and ascorbate can conserve GSH.[19]

Glutathione as Cellular Regulator

That GSH has profound importance for cellular homeostasis and for diverse cellular functions was essentially established by 1978 (see Kosower and Kosower[1] for an excellent review of the early work on GSH). GSH plays a role in such diverse biological processes as protein synthesis, enzyme catalysis, transmembrane transport, receptor action, intermediary metabolism, and cell maturation. Some of the functions in which GSH is involved are illustrated in Fig. 4.

Figure 4. Cellular functions linked to the reducing power of GSH.



Redox phenomena are intrinsic to life processes, and GSH is a major pro-homeostatic modulator of intracellular sulfhydryl (—SH) groups on proteins.[20-22] Many important enzymes (e.g., adenylate cyclase, glucose-6-phosphatase, pyruvate kinase, the transport Ca-ATPases), and at least eight participating in glucose metabolism, are regulatable by redox balance as largely defined by the balance of (2 —SH $\rightleftharpoons$ —S-S—).[21] Other proteins (tubulin of microtubules, thioredoxins,

Table 1. Some of the electron donating capabilities of reduced glutathione (GSH). "•" denotes a single electron generating a radical center. Modified from Bump and Brown.[27]

Hydroxyl radical quenching:
GSH + HO• ⟶ GS• + H2O
Secondary radical quenching:
GSH + R• ⟶ GS• + RH
Quenching of radical centers on DNA:
GSH + DNA• ⟶ GS• + DNA
Quenching of DNA peroxyl radicals:
GSH + DNAOO• ⟶ GS• + DNAOOH
Reduction of lipid peroxides, catalyzed by GSH peroxidases:
2GSH + LOOH ⟶ GSSG + LOH + H2O
Maintenance of protein —SH groups in the reduced state:
2GSH + PSSX ⟶ GSSG + P(SH)2 X
Recycling of vitamin C from its oxidized radical:
2GSH + 2Asc• ⟶ GSSG + 2Asc
Conjugation with P450 products, catalyzed by GSH-S-transferases:
GSH + Substr• ⟶ Substr—GS complex

metallothioneins) have —SH groups at or near their active sites, or are otherwise regulated by the ambient redox state.[20,22] It is evident that glutathione's reducing power is used in conjunction with ascorbate and other antioxidants to protect the entire spectrum of biomolecules, to help regulate their function, and to facilitate the survival and optimal performance of the cell as a living unit.

Glutathione's —SH character and its reducing power also set the redox stage for the proteins known as metallothioneins, which are able to bind with heavy metals and other potential sulfhydryl poisons to facilitate their subsequent removal from the body.[22] Metallothioneins are inducable, and their levels are augmented in response to heavy metal overload or related oxidative challenge.[23]

Glutathione's reducing power is also homeostatically employed to "fine-tune" the redox state of the various cellular environments. For example, the GSH/GSSG ratio is normally very high in mitochondria, and their reducing potential highly negative.[14] This apparently ensures a reducing environment to help control the high flux of oxygen radicals from the mitochondria's OxPhos activities. In the endoplasmic reticulum (ER) there is less endogenous oxidative flux. Also, the protein biosynthesis which is a major activity of the ER does not consistently require a highly reducing environment. Analyses of the ER indicate its GSH/GSSG ratio is low and that the ER micro-environment is set at a comparatively oxidizing point.[24] It seems the GSH/GSSG ratio can be varied in different cell micro-environments, to customize the redox milieu of each for its specialized functions.

GSH Reducing Power Blocks Endogenous Oxidants

Antioxidants are the body's premier resource for protection against the diverse free radical and other oxidative stressors to which it invariably becomes exposed.[25] The antioxidant defense system is sophisticated and adaptive, and GSH is a central constituent of this system.[3] Nowhere is its presence more important than in the mitochondria.

Originating within the mitochondria of aerobic cells is a steady flux of oxygen free radicals, unavoidably generated from the processes that utilize oxygen to make ATP. This complex system of enzyme pathways by which the mitochondria use oxygen to break carbon-carbon bonds and produce ATP is called oxidative phosphorylation (OxPhos). As OxPhos substrates are processed in the mitochondria, invariably single electrons escape, leaking out of the OxPhos complex to react with ambient oxygen and generate oxygen free radicals.[26] This oxygen radical leakage, a type of "metabolic friction" in the aerobic system, both wastes energy and poses a potential toxic risk to the organism. An estimated 2-5 percent of the electrons that pass through the OxPhos system are converted into superoxide and other oxygen radicals.[26] Since

OxPhos processes at least 95 percent of all the oxygen used by the body, this flux of wayward oxygen free radicals is metabolically significant.[3,25,26]

The continual flux of single electrons to oxygen generates an endogenous oxidative stress in human tissues. Superoxide, peroxide, hydroxyl radical, and other free radicals derived from oxygen are highly reactive and therefore threatening to the integrity of essential biomolecules such as DNA and RNA, enzymes and other proteins, and the phospholipids responsible for membrane integrity. The aerobic cell is continually challenged to neutralize these OxyRad time bombs before they can initiate propagative free radical reactions that could cause its disintegration. Healthy cells homeostatically oppose free radicals through the use of antioxidants. Table 1 lists free radical quenching reactions against which GSH can be employed.

With our reliance on oxygen, humans cannot escape this ongoing oxidative challenge. It may be the ultimate challenge of being alive. An ever more impressive body of evidence indicates that the cumulative damaging effects of oxygen radicals and other oxidants are principal contributors to degenerative diseases, and to the progressive loss of organ functions that we recognize as aging.[25]

In the face of this endogenous oxidative burden, the formidable reducing power of the GSH/GSSG couple is a profound physicochemical asset for the aerobic organism. Perhaps equally as significant for lifespan is that GSH also helps protect against exogenous oxidative insults, which are (or ought to be) potentially more controllable.

Glutathione is Depleted by Exogenous Stressors

Oxidative stress originating from outside the body is a feature of life in the modern world. First, the tens of thousands of confirmed toxic substances in our external environment are invariably sources of free radicals or related oxidants. Add to this substantial burden the many negative aspects of the modern, Westernized lifestyle and a picture emerges of the human organism burdened by chronic disease and threatened with a shorter lifespan than might otherwise be possible. The most important of the exogenous oxidative stressors are briefly discussed below.

Cigarette smoke contains thousands of different chemical species, and a single puff of cigarette smoke contains trillions of free radicals.[25] Cigarette smoke literally burns away the antioxidant vitamins C and E, as well as other nutrients. The cigarette tars are long-lived free radical generators and potent carcinogens (reviewed in reference 28).

Many pharmaceutical products are oxidants capable of depleting GSH from the liver, kidneys, heart, and other tissues.[29] The popular over-the-counter drug acetaminophen is a potent oxidant. It depletes GSH from the cells of the liver, and by so doing renders the liver more vulnerable to toxic damage. The anticancer drug Adriamycin has been used in animal experiments as a "model" for free radical-induced tissue damage; its foremost threat is to the heart.[30]

The halogenated hydrocarbons (halocarbons) are potent oxidants. Halocarbons are ubiquitous, being used in the plastics industry, as industrial and dry cleaning solvents, as pesticides and herbicides, and as refrigerants. The chlorofluorocarbons that currently threaten the ozone layer are one type of halocarbon. Halocarbons currently contaminate much of the ground water of the United States, and can now be detected in adipose tissue of humans from around the globe. They are potent free radical generators in the liver, by way of P450 activation, and they effectively deplete liver GSH.[30]

Strenuous aerobic exercise can deplete antioxidants from the skeletal muscles, and

Table 2. Representative substrates of the GSH-S-transferases, in alphabetical order. From Kretschmar and Klinger,[43] and Ketterer, Coles, and Meyer.[44]

Acetaminophen (Tylenol®), other pharmaceuticals
Acetone
Aflatoxin B1, natural toxin from moldy peanuts
Aliphatic halocarbons (vinyl chloride, hexachlorocyclohexane)
Aromatic halocarbons (bromobenzenes, chlorobenzenes)
Benzopyrenes of barbecued foods
Cholesterol, other steroids
Isothiocyanates
Metals (methylmercury, cadmium)
Organophosphate pesticides (methylparathion)
Peroxides, including lipid hydroperoxides generated in vivo
Leukotriene A4 conversion to C4
Prostaglandin H2

sometimes also from the other organs. Exercise increases the body's oxidative burden by calling on the tissues to generate more energy. Making more ATP requires using more oxygen, and this in turn results in greater production of oxygen free radicals. Studies in humans and animals indicate GSH is depleted by exercise, and that for the habitual exerciser supplementation with GSH precursors may be a prudent policy.[31]

Some of the other exogenous factors known to deplete tissue GSH include:

• Dietary deficiency of methionine, an essential amino acid and GSH precursor. The liver uses 70 percent of the total dietary intake.[4]

• Ionizing radiation, whether as X-rays or ultraviolet from sunlight.[32]

• Tissue injury, as from burns,[33] ischemia and reperfusion,[34,37] surgery,[35] septic shock,[4,36] or trauma.[37]

• Iron overload, as in hemochromatosis and transfusional iron excess.[25] Surgery can cause iron release from damaged tissue, and unbound iron catalyzes free radical generation via several putative mechanisms.

• Bacterial or viral infections, including HIV-1.[3,4]

• Alcohol intake is toxic through a number of differing pathways, some of which are free radical/oxidative in character.[38]

Lifestyle choices can be fateful, because negative lifestyle factors (smoking, alcohol consumption, legal or illegal drug use, emotional stress and "life in the fast lane") can converge with environmental stressors to attack the body through related oxidative pathways. Sustained oxidative stress from a heavy cumulative burden of oxidants may deplete the body's GSH and other antioxidant reserves to a point of "dis-stress," beyond which the individual's antioxidant defenses are overwhelmed.[3] The resultant negative antioxidant balance, featuring an excess of free radical challenge over antioxidant defense capabilities, then begins to compromise life functions on a successively wider scale. The cellular consequences of GSH depletion that culminate in cell breakdown and functional failure are outlined in Fig. 5.

The consequences of sustained GSH depletion are grim. As cellular GSH is depleted, first individual cells die in those areas most affected. Then zones of tissue damage begin to appear; those tissues with the highest content of polyunsaturated lipids and/or the most meager antioxidant defenses are generally the most vulnerable. Localized free-radical damage spreads across the tissue in an ever-widening, self-propagating wave. If this spreading wave of tissue degeneration is to be

halted, the antioxidant defenses must be augmented. Repletion of glutathione appears to be central to intrinsic adaptive strategies for meeting the challenge of sustained (or acute) oxidative stress. A discussion of antioxidant adaptation mechanisms is beyond the scope of this review but has been amply discussed elsewhere.[39,40]

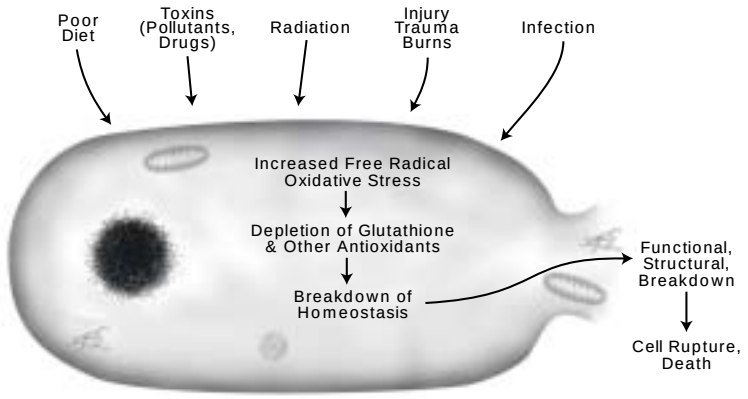
Glutathione As A Systemic Antitoxin: P450 Conjugation

In addition to being a potent free-radical scavenger and ubiquitous enzyme cofactor, GSH is a systemic antitoxin. Normally, GSH is abundant inside cells (at millimolar levels) and relatively lacking outside of cells. One exception is the high concentration of GSH in lower regions of the lungs, where it helps neutralize inhaled toxins (e.g., those from cigarette smoke) and free radicals produced by activated lung phagocytes.[4,25] GSH may be especially important for those organs most directly exposed to exogenous toxins, such as the lungs, the intestines, the kidneys, and particularly the liver.

The liver is the organ most involved with the detoxification of xenobiotics (substances foreign to the body), and also is the main storage locale for GSH (actually exporting GSH to the other organs). Glutathione reaches its highest intracellular concentrations (about 10 millimolar) in the parenchymal cells ("hepatocytes") of the healthy liver. The hepatocytes are highly specialized to synthesize GSH from its precursors or to recycle it from GSSG, as well as to utilize GSH against potential toxicants.[41]

Liver GSH stores are sensitive to depletion by malnutrition or starvation,[42] but in the normally functioning liver the major drain on GSH is the activity of the GSH transferase enzymes (GSTs).[41] These are a large family of cytosolic isozymes with a collective broad specificity for endogenous "orthomolecules" (molecules orthodox to the body) as well as for xenobiotics. They are inducable, meaning they are synthesized in higher quantities in response to challenge. Exhibiting multiple forms, and differing in their developmental patterns and inducabilities, the GSTs constitute 10 percent of the extractable protein of rat liver. In classic toxicology, these are the "P450 Phase II" conjugating enzymes.

Figure 5. Cell Breakdown related to depletion of GSH. From Kidd and Huber.[3]



The GSTs have relatively broad specificity for their substrates, but absolute specificity for GSH as their electron-donating cofactor. Table 2 lists some of the major GST substrates.

The role of GSH in liver P450 conjugation activity normally is quite considerable, accounting for up to 60% of all the liver metabolites found in bile. But while GSH conjugation is unquestionably of net benefit to the organism, its outcome is not positive in every instance. Thus, several classes of xenobiotics induce or otherwise activate P450-type enzymes, which generate GSH conjugates that are then more potentially toxic than the parent xenobiotic.[45]

Factors that deplete the liver pool of GSH can decrease conjugation and increase the toxicity of xenobiotics. As examples, experimental acetaminophen (Tylenol®) toxicity is markedly enhanced after a 48-hour fast, as is the toxicity of bromobenzene, a halogenated aromatic hydrocarbon.[30] Both these xenobiotics have been used in animal experiments as model GSH depletors, being predictably transformed by the liver P450 system into free-radical metabolites. A "titration" type of experiment can be done with rats, in which liver GSH is depleted by exposure to acetaminophen or bromobenzene, and liver damage ensues. The damage can be ameliorated by increasing the liver's reserves of glutathione, and is made worse by prior depletion of the liver's GSH reserves (as by withholding food prior to conducting the experiment). Also, prior depletion of liver GSH by one xenobiotic can predispose the liver to damage by another GSH-depleting oxidant upon subsequent exposure. One well-documented example is concurrent dosing with alcohol and certain pharmaceuticals.[38]

It is highly doubtful that the P450 activities of the liver evolved in order to metabolize the tens of thousands of petrochemical derivatives and other xenobiotics that are delivered to the human liver by the modern environment. Rather, the P450 system seems geared primarily to effect homeostatic control over circulating steroid hormones and other endogenous, fat-soluble substances. Under this scenario, despite GSH having the antioxidant versatility that it does, the enzyme-catalyzed, Phase II conjugation of xenobiotics with GSH does not guarantee freedom from toxic damage.

To optimize nutritional support for the liver's detoxification functions, it is more rational to supply other nutrients in addition to GSH. A variety of oral antioxidants would be required for support of the entire antioxidant defense system (including its GSH branches), and non-antioxidant nutrients (phosphatidylcholine, for example[46] and B vitamins and minerals) would lend additional dimensions of support. Short of the total elimination of xenobiotics from the planetary environment, rational dietary supplementation is our best bet for coping with this exogenous oxidant stress.

Glutathione Deficiency in Liver Diseases

GSH depletion has been suggested to represent an important contributory factor to liver injury, and to enhanced morbidity related to liver hypofunction[4]. In one small study, subnormal plasma concentrations of GSH were observed in cirrhosis patients, independent of their diet.[47] A larger study demonstrated a four- to eight-fold decrease in plasma GSH in 48 cirrhotic patients versus 18 healthy volunteers.[48] A significant decrease in cysteine in severe cirrhosis also was observed.

Altomare and collaborators measured liver GSH and GSSG in chronic alcoholics, in patients with nonalcoholic liver diseases (fatty liver, acute and chronic hepatitis, cirrhosis), and control patients (admitted for uncomplicated abdominal procedures).[49] They found GSH decreased in the alcoholic and nonalcoholic liver disease groups, compared with the control groups; GSSG was also significantly higher in these groups. The investigators postulated that decreased GSH and/or increased GSSG could have contributed to liver injury susceptibility and toxic risk in these patients, while altering fundamental cell functions such as protein synthesis, enzyme activities, transport processes, microtubular and other structural support, and secretion mechanisms. Other studies also have documented plasma and liver GSH decreases in patients with acute viral hepatitis, and in chronic cases of hepatitis, alcoholic liver disease, or nonalcoholic cirrhosis.[50,51]

Deficiency of GSH caused by one toxin may render the liver more vulnerable to

other toxins. One example is acetaminophen intake superimposed on the alcohol-damaged liver.[51] In a group of chronic alcoholics with GSH deficiency, acetaminophen did not lower GSH unless gamma-glutamyl transferase (SGGT) was high to begin with. Those subjects with abnormally elevated SGGT manifested abnormally lowered plasma GSH after acetaminophen intake, and were therefore more predisposed to further liver damage from other toxic agents.

Glutathione and Lung Diseases

Being directly in the path of airborne materials, the lung tissue is particularly at risk from oxidative stressors such as cigarette smoke, atmospheric pollutants, and other inhaled environmental toxins.[28] GSH and GSH-associated enzymes present in the epithelial lining fluid (ELF) of the lower respiratory tract may be the first line of defense against such challenges.[41,52,53] Sustained oxidative challenge to the lung results in depletion of GSH and other antioxidants from the lungs.

GSH deficiencies have been documented in a number of pulmonary diseases, including acute respiratory distress syndrome (ARDS), asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and neonatal lung damage.[4] Patients with ARDS and sepsis have a deficiency of GSH in the ELF as compared with healthy subjects,[52,53] and a greater percentage of the total ELF glutathione is in the oxidized form (GSSG), indicating increased oxidative stress in the lower respiratory tract.[53] When GSH was repleted in their ELF using intravenous N-acetylcysteine, patients in intensive care regained independent lung function and left the intensive care unit significantly faster.[54]

Airway inflammation in asthma also features increased generation of free radical oxidants. As earlier indicated from animal experiments, subjects with mild asthma seemingly have the capacity to adaptively increase their antioxidant defenses, as manifested in their alveolar GSH concentrations being significantly higher than healthy volunteers.[55] By contrast, in patients with idiopathic pulmonary fibrosis, GSH concentrations in the ELF are a mere 25% of normal, and may contribute to the pathophysiology of this disease.[41]

Infants born prematurely at 25 weeks average gestational age were found to have significantly lower pulmonary GSH than did infants born at 40 weeks.[56] Among infants born at 35 weeks, those with lower GSH levels in their ELF were found more susceptible to subsequent chronic lung disease. These findings suggest that poor lung GSH status at birth may predispose the infant to respiratory pathologies.

Glutathione, Immunity, and HIV Disease

As with other cell types, the proliferation, growth, and differentiation of immune cells is dependent on GSH. Both the T and the B lymphocytes require adequate levels of intracellular GSH to differentiate, and healthy humans with relatively low lymphocyte GSH were found to have significantly lower CD4 counts.[57] Intracellular GSH is also required for the T-cell proliferative response to mitogenic stimulation, for the activation of cytotoxic T "killer" cells,[58] and for many specific T-cell functions, including DNA synthesis for cell replication, as well as for the metabolism of interleukin-2 which is important for the mitogenic response.[59]

Experimental depletion of GSH inhibits immune cell functions, sometimes markedly,[58,60] and in a number of different experimental systems the intracellular GSH of lymphocytes was shown to determine the magnitude of immunological capacity.[58] These and other findings indicate that intracellular GSH status plays a central role in the functioning of immune cells.

In the auto-immune diseases of systemic lupus erythematosis (SLE) and rheumatoid arthritis (RA), and as seen in aging, T lymphocytes demonstrate depressed responsiveness to antigens and mitogens, perhaps because of insufficient IL-2 production (see reference 60 for a review). Patients with RA had low blood sulfhydryl (—SH) status,[60] as did patients with Type II diabetes or with ulcerative colitis.[13,60]

Chronic viral infections may trigger GSH depletion in circulating immune cells or GSH/GSSG imbalance. Patients chronically infected with the hepatitis C virus were found to have low GSH in their circulating monocytes.[13] Monocyte GSH levels were abnormal in early HIV-1 disease,[61] then in patients with advanced disease the GSH levels normalized in monocytes but the GSH/GSSG ratio became abnormal. Significant decreases in the plasma levels of both cysteine and cystine also were documented in subjects with HIV-1 infection.[61-63] Since cysteine is a rate-limiting precursor for GSH synthesis, an associated decrease of GSH in the lung ELF was highly suggestive of a systemic GSH insufficiency in these subjects.[64] The most marked GSH decreases occurred in subjects who were asymptomatic but had CD-4 counts below 400. Both the abnormal cytokine expression and the progression to weight loss seen in HIV-1 disease may be linked (at least in part) to abnormalities in the uptake of GSH precursors by immune cells of HIV-1 subjects, and/or to abnormalities in their synthesis of GSH.

Neurodegeneration Related to Glutathione Depletion

Perhaps the most challenging aspect of the clinical research on free radicals and antioxidants has been to relate oxidative stress to disease causality. Thus, GSH depletion has been hard to position as "the smoking gun" at the scene of the "crime." However, few experts in this field seriously continue to doubt that free-radical propagation and associated antioxidant depletion are involved in at least some types of degenerative tissue breakdown.[25] Numerous studies link free-radical damage with degenerative brain conditions.

The brain is particularly susceptible to free radical attack: it is highly oxygenated, which makes it vulnerable to endogenous oxygen radical production, and it has a high proportion of unsaturated lipid which makes it vulnerable to peroxidation. In addition, those brain regions that are rich in catecholamines are exceptionally vulnerable to free radical generation. The catecholamines adrenaline, noradrenaline, and dopamine can spontaneously break down (auto-oxidize) to free radicals, or become metabolized to radicals by the endogenous enzymes known as MAO — the monoamine oxidases.[65,66] One such region is the substantia nigra (SN), where a connection has been established between antioxidant depletion (including GSH) and tissue degeneration.

Parkinson's Disease (PD) is based primarily in the SN. It is to date the most suggestive example of likely causality of oxidative stress in neural degeneration. Lipid peroxidation had been reported increased in this condition, although causality was not established;[67] then studies found GSH levels were dramatically decreased in PD.[68,69] Jenner et al[69] suggested GSH depletion might have particular significance in PD, especially since such depletion often predates the emergence of clinical symptoms.

The melanized catecholaminergic cells found in large quantities in the SN contain less GSH peroxidase and tend to bind to redox-active metals, which makes them more vulnerable to free radical generation from their easily oxidizable melanin complement. Several studies have demonstrated increased levels of such metals, especially iron, in PD brains compared with controls (reviewed by Lohr and Browning[67]).

Several antioxidants have been measured decreased in PD tissue.[4,69] Indicators such as the disappearance of melanin from the SN, the increase of total iron and ferric iron, the marked decrease of GSH in the SN, the decreases in antioxidant enzyme activities, and the substantial increases of lipid peroxidation indicators, all argue for oxidative stress playing a role in the initiation and/or progression of PD.[69] In a study using high-dose antioxidants, Fahn[70] found that a combination of vitamin E (3,200 IU per day) and vitamin C (3,000 mg per day) could slow PD progression. Further controlled studies are needed that involve GSH precursors, administered preferably in combination with other antioxidants.

Tardive dyskinesia (TD), a movement disorder centered in the basal ganglia, has been linked to long-term treatment with neuroleptic drugs. The basal ganglia are exceptionally vulnerable to free-radical overload because they are so rich in dopamine as well as other catecholamines. By blocking dopamine receptors, neuroleptics may cause dopamine buildup in the basal ganglia that then increases free-radical production. Glutamate excess may also contribute to the free-radical overload in TD. Lohr and co-workers[67] also found elevated lipid peroxide levels in the cerebrospinal fluid of patients maintained on neuroleptics and exhibiting symptoms of TD. They succeeded in decreasing the severity of TD using high doses of vitamin E, and called for further trials with combinations of antioxidants.

Schizophrenia may have a component of free-radical overload. Lipid peroxides have been found elevated in the blood, and Phillips and co-workers found increased pentane gas, a marker for lipid peroxidation, in the breath of schizophrenics as compared with normal volunteers and with patients having other psychiatric illness.[71] The enzyme SOD (superoxide dismutase, which metabolizes superoxide radicals) was found increased, possibly as an adaptive response to free radical overload. Studies of antioxidant treatment in schizophrenia have been few; two recent studies that examined only vitamin C yielded conflicting results.[67] Especially since GSH peroxidase was also found to be reduced,[72] future trials with antioxidants in schizophrenia should include selenium and GSH precursor nutrients.

Down's Syndrome (DS), the classic mental deficiency disease resulting from a trisomy of chromosome 21, is known to feature increased systemic oxidative stress.[39] The 50% overexpression of SOD on chromosome 21 contributes to heightened fluxes of superoxide in all the tissues. Yet DS does not manifest until after birth; the mother's antioxidant defenses may protect the fetus until delivery. Reportedly, parents have experienced success with nutritional antioxidants in conserving their DS children's mental resources after birth.[73] DS children are also at greatly increased risk for an Alzheimer's-type dementia as they age,[67] and it should prove exciting to determine whether potent nutritional supplementation from birth can delay the onset of dementia in DS subjects.

Alzheimer's Disease (AD), though almost certainly multifactorial in its etiology, has both direct and indirect indications of free radical involvement. Increased lipid peroxides have been reported from the temporal and cerebral cortex of patients with AD as compared with controls.[67] Jenner[69] reported that iron was raised and GSH was decreased in the cortical areas; and Richardson and co-investigators[74] added iron to homogenates of frontal cortex from AD patients and found significantly higher lipid peroxide generation. Fibroblast cells cultured from patients with AD exhibited increased susceptibility to free-radical damage over controls; the sites of their increased vulnerability may be the mitochondria.[67] Glutathione metabolism may also be abnormal in AD; Adams and co-investigators[68] found GSH to be lower in the

hippocampus, the primary site of short-term memory initiation, and Jenner[69] found that GSH was decreased in the cortical areas.

The evidence to date for possible oxidative stress in DS, PD, TD, schizophrenia and AD is suggestive, if not yet strongly persuasive. As pointed out by Jenner,[69] if oxidative stress does contribute to neural degeneration, whether it is eventually proven to be primary or secondary in the etiologic progression, the therapeutic rewards are likely to be great. Future trials are indicated with dietary GSH precursors, administered in combination with other antioxidants, antioxidant cofactors, and non-antioxidant brain-trophic nutrients such as phosphatidylserine.

Glutathione, Atherosclerosis, and Prostaglandins

The endothelial cells that line the blood vessel lumina are arranged in a single, attenuated layer, and are vulnerable to oxidative challenge. They are continually exposed both to exogenous oxidants that reach the circulation, and to endogenous sources of oxidative challenge such as hydrogen peroxide (produced from OxPhos fluxes) or activated phagocytic cells. Atherosclerosis appears linked to oxidative damage to the vessel wall. Increased lipid peroxides, decreased GSH peroxidase levels, and lowered levels of the protective eicosanoid prostacyclin (PGI2) have been documented in human and animal atherosclerotic arteries.[75] Oxidative stress within atherosclerotic arteries depletes GSH and other antioxidants, and results in a shift in the so-called "prostaglandin" (more correctly, eicosanoid) balance from anti-inflammatory towards pro-inflammatory.[76]

Both the GSH peroxidase enzymes and various GSH-S-transferases may be employed in the endothelia for "yin-yang" regulation of vascular tone and responsiveness, mainly through their influences on eicosanoid balance; the more active they are, the better the production of protective eicosanoids. GSH can produce coronary vasodilation when added to isolated, perfused rodent heart, very likely due to its normalizing effect on prostaglandin synthesis.[77]

Platelets, like other tissues, contain millimolar levels of GSH. The main interactions studied to date between GSH, platelets, and the arterial wall have to do with the leukotriene and prostaglandin eicosanoids.[78] Oxidative stressors tend to shift the platelet's eicosanoid balance away from PGI2 and toward thromboxane (TxA2), resulting in a proaggregatory state.

Platelets from diabetics have lower GSH levels and make excess TxA2, thus having a lowered threshold for aggregation; this may contribute to the increased atherosclerosis seen in the diabetic population.[75] Reduced levels of GSH peroxidase have been found in the platelets of patients with acute myocardial infarction, Glanzmann's thrombasthenia, and the Hermansky-Pudlak syndrome characterized by dysfunctional platelets.[75] Exogenous GSH or combinations of antioxidants can be employed to raise the threshold for platelet aggregation, and so ultimately to protect the endothelium against further damage.[78,79]

Glutathione Abnormalities in Other Conditions

Human pancreatic inflammatory states, whether acute, acute recurrent, or chronic, have been linked to damage inflicted on the pancreatic tissue by oxygen free radicals.[80] Concomitantly, these patients suffered from a depletion of antioxidants. Many showed increased lipid peroxidation products in their pancreatic tissue, duodenal juice, and bile. After evidence of GSH over-oxidation (GSSG excess) in patients admitted to hospital with alcohol-provoked relapse of pancreatitis, one patient was treated with N-acetylcysteine (NAC), a precursor of GSH.

Within 72 hours, the patient had improved significantly. This prompted a preliminary randomized trial of NAC on patients suffering from acute pancreatitis. Clinical status was significantly better on the second and third day in those patients with combined pancreatic and other organ failure who were treated with NAC.[81]

Chronic pancreatitis patients also have shown increased serum lipid peroxides, with those in relapse generally showing the greater increases.[82] Such patients often were deficient in several antioxidants. Uden and collaborators[83] did a small double-blind, crossover trial in which they gave selenium, vitamin A, vitamins C and E, and methionine (a cysteine precursor) to patients with pancreatitis (mixed acute and chronic). This therapy significantly reduced pain and prevented relapse, independent of the etiology and acuteness of the disease. Larger trials are needed, but to date supplementation with mixed antioxidants appears promising in pancreatic inflammatory states.

Metal storage diseases have become another area of focus for GSH and other antioxidant therapies. Both hepatic iron overload and copper overload feature increased lipid peroxidation and detectable free radical damage at the cell level.[84] Humans with thalassemia and secondary iron overload showed significant reduction in GSH reductase activity. Summer and Eisenburg examined copper-overloaded (Wilson's Disease) patients,[85] and found hepatic GSH markedly lower in biopsies of five out of six patients as compared with controls. Despite an impressive body of animal data indicating antioxidant depletion in iron and copper overload states, no randomized controlled trials have yet been conducted on humans.

Sickle cell anemia is a chronic hereditary anemia in which the lifespan of the red cell is markedly decreased, from an average 120 days to 17 days. Abnormal rod-like fibers of hemoglobin in the red cell cause an irreversible transition to a sickle shape, and "sickling crises" can be life-threatening. Sickling is associated with increased oxidative stress in the red cell, and depletion of antioxidants has been reported, including GSH.[86]

Glutathione in Aging

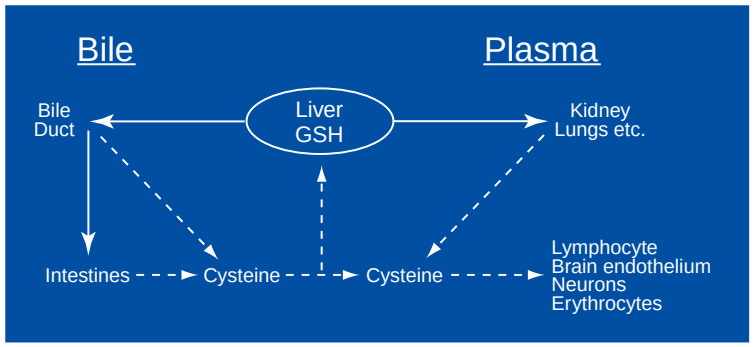
Studies on GSH status with advancing age have been few, but to date there does appear to be a correlation between age-associated GSH depletion and poor health. Lang and collaborators[87] compared blood GSH concentrations between the healthy young and healthy elderly subjects. The 40 young subjects (20-39 years of age) had a blood GSH level 17% higher on average than the 60 elderly subjects (60-79 years). Julius et al[88] measured GSH in 33 persons of ages 60-79 years. Higher GSH concentrations were associated with good health, regardless of age; subjects with chronic diseases had lower mean GSH concentrations than those free of disease. Further studies should clarify whether systemic GSH status is indeed a predictor of good health with advancing age.

Strategies for Repleting Cellular Glutathione

In light of the copious evidence supporting the importance of GSH for homeostasis, and for resistance to toxic attack, implementation of measures aimed at increasing cellular GSH would seem prudent. Optimizing GSH would likely augment antioxidant defenses, and stabilize or raise the cell's threshold for susceptibility to toxic attack. The first possible measure to consider would be oral dosing with GSH.

Glutathione given orally does raise GSH *in vivo* — this has been demonstrated both in animals and in humans.[4] In one study, an oral bolus of 15 mg/kg to the human appears to raise plasma GSH two- to five-fold,[89] with

Figure 6. Interorgan homeostasis of GSH and cysteine. From Kaplowitz et al.[95]



great variability in effect between the five subjects tested. Equivalent amounts of individual amino acid precursors of GSH failed to raise plasma GSH above baseline. In another study that used healthy, fasted subjects, plasma GSH did not rise following oral administration of GSH.[91] Perhaps plasma GSH is so well buffered in healthy subjects that it is difficult to influence by oral dosing.

The enterocyte cells that line the intestinal lumen absorb GSH via non-energy-requiring, carrier-mediated diffusion, and later export it into the blood.[4] GSH also can be absorbed intact by epithelial cells other than the enterocytes, such as lung alveolar cells, vessel endothelial cells, retinal pigmented epithelial cells, and cells of the kidney's proximal tubule; it seems also to cross the blood-brain barrier. Intact GSH also can be delivered directly into the lungs as an aerosol.[90] Other cells — brain endothelial and nerve cells, red blood cells, lymphocytes — appear incapable of absorbing GSH as the intact tripeptide; rather they must synthesize GSH anew from cysteine (or cystine) that they transport inward from the outside.[4] Here transpeptidase enzymes on the outside surface of the cell assist by removing single amino acids from circulating GSH, some of which are then subsequently absorbed (refer to Fig. 2). Thus, administering GSH as the whole molecule may be worthwhile as a means to directly replete GSH in the intestinal lining cells or other epithelia *in vivo*; otherwise, it is not a particularly cost-effective way to accomplish GSH repletion.

Another dietary means for repleting GSH is the amino acid L-methionine. This is an essential amino acid, so it must be obtained from the diet. But methionine is metabolically upstream: it must first be converted to cysteine which itself is then available for synthesis into GSH. This pathway requires many co-factors and may be inactive in neonates and in certain adults, such as patients with liver disease.[4] The "activated" methionine metabolite known as SAM (S-adenosyl methionine) is effective in raising red cell GSH and hepatic GSH when given orally at 1600 mg per day.[4] SAM has proven clinical benefit against cirrhosis and cholestasis,[92] but is not commercially available in the United States.

The sulfur-containing amino acid L-cysteine is the precursor that most limits the synthesis of GSH. When substituted into the diet in place of the total protein allowance it was just as effective in repleting GSH levels.[93] But cysteine is probably unsafe for routine oral administration. When circulating in the blood it readily auto-oxidizes to potentially toxic degradation products. Saez and collaborators demonstrated that the highly reactive hydroxyl radical is among the products formed from the auto-oxidation of cysteine.[94] Cysteine also has "excitotoxin" activity in the brain, similar to that of the amino acids glutamate and aspartate, and can be toxic to the retina. GSH has none of these liabilities, and the GSH redox system may have evolved to supplant the relatively fallible, cysteine-based system.

It has been suggested that GSH acts *in vivo* as a reservoir for cysteine.[93,94] Cysteine is unstable in the blood because the ambient oxygen is high enough to oxidize it, yet its availability limits GSH synthesis. The cystine produced from cysteine oxidation is not significantly taken up into cells other than those of the kidney, and requires energy and enzymatic intervention to be converted to cysteine. The mechanistic solution to this

problem may be that once replete with GSH, the liver's cells "export" it (Fig. 6). After GSH exits the liver cell, it can quickly be back-converted to cysteine, which then is used elsewhere for protein synthesis and for the biosynthesis of taurine and other sulfur metabolites. Circulating GSH is safe; it reacts only slowly with oxygen, is less susceptible to auto-oxidative degradation than is cysteine, and is more soluble in the plasma. Certainly as a water-soluble, transportable form of sulfhydryl (—SH) reducing power, GSH is more reliable than circulating cysteine.

Some GSH comes in with the diet (150 mg daily by rough estimate),[4] but the majority of the body's GSH is made in the liver. Liver GSH synthesis is closely linked to overall protein synthesis, and also to intakes of sulfur amino acids from the diet.[93] The body's other organs seem to draw on GSH exported from the liver, by way of the circulation as well as the bile. Hormones and other vasoactive substances increase GSH efflux into the bile, and this may contribute to the hepatic GSH loss noted under conditions of stress.[41] About 80 percent of the GSH synthesized in the liver is exported from the hepatocytes, and most of this is utilized by the kidneys, which also carry a major toxic burden.[41] Keeping the liver replete with GSH provides the body with a reservoir of GSH and sulfhydryl reducing power for its systemic detoxification needs, and makes for extra capacity to supply cysteine, taurine, and other sulfur amino acids as needed. It accomplishes all this while simultaneously conserving the essential amino acid methionine for other applications.

With some cells of the body unable to directly utilize GSH, with cysteine's availability being the main factor limiting GSH synthesis in the cells, and with dietary L-cysteine known to be potentially toxic, N-acetyl cysteine (NAC) takes on important significance as a dietary GSH source. NAC is a cysteine precursor; it is well absorbed by the intestine, and becomes converted to circulating cysteine by de-acetylation. It seems not to raise GSH levels if they are already within the normal range, but it can raise abnormally low GSH levels back to normal. This is the basis for its use as an antidote to acetaminophen's liver toxicity.[29,96]

Used in Europe for many years as a mucolytic agent, NAC has antimutagenic and anticarcinogenic properties while also being a potent antioxidant. It may not be the perfect GSH source. In a clinical study van Zandwijk found that a daily dose of 600 mg was beneficial and innocuous while 1200 mg and 1800 mg per day caused significant adverse effects.[97] Yet Cathcart has observed negligible side effects with NAC intakes much higher than this level in his HIV-1/AIDs patients since 1985.[98]

Other synthetic, oral delivery sources of GSH have been developed.[13] The compound L-2-oxothiazolidine-4-carboxylate (OTC) is a substrate for the enzyme 5-oxoprolinase, which converts it to S-carboxy cysteine; this then hydrolyzes to yield cysteine, which becomes incorporated into GSH within liver cells. But the needed enzyme is not found in all the tissues, and it is not clear that OTC can consistently increase GSH on a systemic basis.

Glutathione esters, synthetic compounds prepared by linking the glycyl end of GSH into ester bonds, have been the subject of much research by Meister, Anderson, and their associates[12-14] as potential oral GSH delivery compounds. These esters do appear to be effective GSH delivery vehicles, but have the disadvantage that all yield alcohols *in vivo* when their ester bonds are broken, and their safety over the long term has yet to be satisfactorily demonstrated. Occasional reports of their toxicity have so far been blamed on metal impurities.[13]

A list of GSH precursors with known safety profiles would include NAC, as well as glycine, L-glutamine, L-taurine, L-methionine, and S-adenosyl methionine; L-cysteine should be avoided.

Conclusion

Glutathione is a significant component of the collective antioxidant defenses, and a highly potent antioxidant and antitoxin in its own right. The —SH group of GSH is important for many facets of cell function, and early suggestions that GSH plays multiple regulatory roles at the cell level[1] are borne out by the cumulative findings. Observations from hereditary GSH synthesis deficiencies confirm that GSH is essential both to the functionality and to the structural integrity of the cells, the tissues, and the organ systems. The glutathione status of a cell (that is, the excess of reduced over oxidized glutathione) will perhaps turn out to be the most accurate single indicator of the health of the cell. That is, as glutathione levels go, so will go the fortunes of the cell.

The mitochondria may be the Achilles Heel of the aerobic cell, and mitochondrial breakdown could be the common etiologic thread in most (if not all) GSH deficiency states. The mitochondria are exposed to oxygen free radicals produced by the OxPhos processes, yet cannot make their own GSH for protection — they must expend energy to import it from the surrounding cellular cytosol. The mitochondria do have antioxidant protective enzymes that are inducable (including superoxide dismutase and catalase, but GSH peroxidase demands GSH as cofactor), but this adaptive capacity has its limits. Healthy mitochondria avidly conserve their GSH, but as cytosolic GSH levels decrease, mitochondrial GSH can fall below a critical threshold. The turning point is when, in the face of sustained oxidative challenge, the mitochondrial GSH becomes depleted. The membrane-associated enzymes that transport GSH into the mitochondria then sustain damage, and GSH import is dealt a fatal blow. As a consequence, the mitochondria become casualties of their own making, i.e., destroyed by their own endogenously-generated free radicals.

The consistent findings of GSH depletion in many preclinical and clinical degenerative conditions beg the question of whether antioxidants should be universally employed—whether singly or in combination—in efforts to ameliorate functional degeneration and improve quality of life. Combinations of antioxidants given as dietary supplements seem to offer the most promise for achieving clinical breakthroughs. At times, the administration of massive amounts of ascorbate (orally or intravenously) or of sulfhydryls (GSH and NAC orally and intravenously) will be lifesaving.

Prenatal diagnosis of inherited GSH abnormalities may not be far off.[11] In the meantime, dietary repletion of systemic GSH holds promise for the management of conditions as diverse as Alzheimer's Disease, atherosclerotic vascular degeneration, cataract, lung insufficiencies, Parkinson's Disease, and many others. Assiduous attention to repletion of GSH also should help assist the body to manage bouts of heavy exercise or combat a chronic viral load. Particularly when employed in conjunction with ascorbate, other antioxidants, and other nutritional factors, the reducing power of GSH is a powerful orthomolecular tool for quality and length of life.

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