Glutathione in disease

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Glutathione and disease

GSH is the most abundant intracellular non-protein thiol and has been implicated in many cellular functions including detoxification, synthesis, cell-cycle regulation, regulation of gene expression, protection of proteins and other cellular components, and as an antioxidant [1-4]. It can scavenge free radicals and electrophiles non-enzymically, as well as detoxify xenobiotics via the glutathione S-transferase reaction. It reduces lipid peroxides and hydrogen peroxide via glutathione peroxidase. Oxidation converts it to the disulphide form (GSSG).

As a tripeptide, γ-glutamylcysteinylglycine, it is synthesized from its constituent amino acids in two steps requiring ATP. The first step is catalysed by γ-glutamylcysteine synthetase (γ-GCS) and is rate-determining. This enzyme is inhibited by the final product GSH exerting feedback control. Thus when tissue GSH levels fall, the inhibition of γ-GCS is released promoting further synthesis of GSH. Often GSH synthesis is limited by the supply of free cysteine, present at very low concentrations in plasma.

It would be wasteful if GSH were a single-shot antioxidant so after conversion to the oxidized form (GSSG), it is recycled via the glutathione reductase (GR) reaction (Figure 1). This reaction requires NADPH mostly produced in the initial steps of the pentose phosphate pathway. If this system is overloaded and GSSG accumulates it will form disulphides with proteins (PSSG), and it can be expelled from the cell. Raised GSSG levels will also inactivate thiol enzymes. Oxidative stress increases GSSG and may initially deplete GSH, but a number of adaptive responses, including increased uptake of cysteine, glutamate and glycine together with induction of GR and glutathione peroxidase, may eventually lead to elevated concentrations of GSH [1].

Clearly, with its multiple roles in normal tissues and its complex systems for synthesis and regeneration there is considerable potential for changes in GSH to be associated with disease whether as part of the causal pathways or simply as a result of the disease processes, and indeed low levels of GSH have been reported in aging and various diseases. For example, GSH levels decrease with age in human lens [5], mouse erythrocyte, liver, kidney and heart [6-8]; however, a steady increase in GSH with age was reported in mouse brain stem [9].

The lowered GSH levels may partly explain the altered immunity and increased risk of neoplasia seen with increasing age [2]. The reduced capacity to detoxify could provide a toxicological basis for aging [8]. This could be extended from

Figure 1
Oxidation and regeneration of GSH
PSSG, protein/GSH mixed disulphide; PSH, protein thiol; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; PPP, pentose phosphate pathway.

Abbreviations used: G6PD, glucose-6-phosphate dehydrogenase; GR, glutathione reductase; 6PGD, 6-phosphogluconate dehydrogenase; PSH, protein thiol; PSSG, protein/GSH mixed disulphides; γ-GCS, γ-glutamylcysteine synthase.
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detoxification to prevention of harmful non-enzymic modifications of proteins by glycation, carbamylation and similar reactions. GSH decreases the rate of reaction of sugars with proteins [10,11].

Lowered levels of GSH have been reported in the blood of subjects with arthritis, diabetes or heart disease [12], in liver and other tissues in diabetes [13], and in human cataract lenses, especially those from diabetic subjects [5,14]. The lens, which lacks both protein synthesis and oxidative phosphorylation in most of its cells, has particular problems in maintaining levels of GSH. There are many animal models of cataract, and GSH levels are decreased in at least 22 of them, demonstrating a major role for this molecule in maintaining lens function [4].

GSH levels may be lowered by certain drugs, for example valproic acid, an anticonvulsant [15]. On the positive side, higher blood GSH levels in elderly subjects were associated with fewer illnesses and a higher self-rated health score [12].

Lowered GSH levels in tissues could occur as a result of decreased synthesis, increased breakdown, oxidation to GSSG and mixed disulphides, failure to regenerate GSH from GSSG, leakage, extrusion, its use in detoxification reactions or by a combination of these routes (Table 1). Each of these represents a set of underlying causes.

Decreased synthesis could be related to poor nutrition via decreased cysteine levels available to tissues. Any deficiency in cysteine uptake would also limit synthesis. Impediment of GSH transport [16,17] would have different effects depending on the tissue. A lack of ATP would decrease GSH synthesis, which requires two ATP molecules per GSH synthesized and is a major consumer of ATP. Decreased synthesis could also result from low activity of the two enzymes in the synthetic pathway. Subjects with an inherited deficiency of γ-GCS have extremely low red blood cell GSH concentrations and a non-spherocytic haemolytic anaemia [2]. GSH synthetase deficiency is associated with a milder haemolytic disorder.

The role of GSH as an antioxidant, by both enzymic and non-enzymic routes, leads to the formation of GSSG and PSSG. PSSG accumulate in human cataract [5] and diabetic cataract [18]. Accumulation of GSSG is normally prevented by its regeneration in the GR reaction but regeneration would be compromised if GR or NADPH levels fell. GR activity falls in some human cataracts [4], in X-ray cataract in rabbit [19], in various tissues in diabetes [13] and in testicular tumours [20]. The decline in GR activity in aging erythrocytes parallels the fall in GSH [6].

Curiously, an elevated activity of GR has been reported in the amygdala and hippocampus of patients with Alzheimer's disease [21]. It decreases in epileptic children on valproic acid therapy [15]. Subjects with an almost complete absence of GR from their red blood cells had normal GSH levels in the cells; nevertheless cataract developed [2]. It appears that GR is not especially important in maintaining normal GSH levels but may become vital when the GSH system is stressed oxidatively or otherwise. A proposed role for GR in human cataract is undermined by the finding that normal human lens has vastly more GR than those of common laboratory or domestic species [22] or more exotic species [23]. The late fall in GR may be a consequence of the cataractogenic process rather than a cause.

The failure to regenerate GSH from GSSG in the GR reaction could also be due to a lack of NADPH. This is mostly produced from the G6PD and 6PGD reactions of the pentose phosphate pathway (Figure 1). Patients with G6PD deficiency are susceptible to a rapid fall in erythrocyte GSH when exposed to oxidants, resulting in a haemolytic crisis [2]. G6PD activity decreases in the lens with age [24,25] and in cataracts [4,26,27].

Failure of any of the systems that help to maintain GSH levels (Table 1) could lead to its decline. Declining activity of the enzymes involved is of particular interest and may well involve non-enzymic modification by sugars, cyanate or other small molecules found in all cells.

### Table 1

<table>
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<tr>
<th>Possible causes of low GSH</th>
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<tbody>
<tr>
<td>Decreased synthesis</td>
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<tr>
<td>- Lack of cysteine</td>
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<tr>
<td>- Lack of ATP</td>
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<tr>
<td>- Lack of the two enzymes (especially γ-GCS)</td>
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<tr>
<td>Increased breakdown</td>
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<tr>
<td>- Oxidation to GSSG and PSSG</td>
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<tr>
<td>Failure to regenerate GSH from GSSG</td>
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<tr>
<td>- Lack of NADPH</td>
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<tr>
<td>- Lowered activity of GR</td>
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<tr>
<td>Leakage/extrusion</td>
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<tr>
<td>Use in detoxification (GSH S-transferase)</td>
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These reactions have been studied in great detail in relation to structural proteins in aging and diabetes [28], but being non-enzymic they are also not specific so that any protein will be susceptible. In tissues like liver, protein turnover is so rapid that modified proteins, including enzymes, are swiftly eliminated. In other tissues, especially the lens, damaged proteins accumulate. For example, the activity of G6PD and superoxide dismutase declines in the centre of the lens but the inactive proteins can be detected immunologically [24,29]. Recently, attention has turned to inactivation of enzymes by reaction with sugars and cyanate to provide insight into what might occur in tissues in aging, diabetes, renal failure and cataract.

G6PD is inactivated by carbamylation, with even low concentrations of cyanate (10 mM) inducing a 25–30% inactivation over 3–6 h (E. Ganea and J. J. Harding, unpublished work).

GR is slowly inactivated by incubation with glucose, glucose 6-phosphate and fructose [30]. This might in part account for the decreased activity seen in diabetes [13,31,32] and in human cataract [4] and thus for the decreased GSH. The sugars inactivate in the order of their glycation activity [33]. It is beginning to emerge that glycation under relatively mild conditions can inactivate a number of enzymes ([34]; J. J. Harding, K. C. Rixon, M. Wilson and D. Jones, unpublished work). Inactivation of enzymes of GSH metabolism could lead to lowering of GSH levels in tissues (Figure 2). Aspirin, a known antiglycation agent, provides some protection against the glycation-induced inactivation of GR [30]. A more dramatic protection is afforded by α-crystallin, a major lens protein which has now been identified in a wide variety of tissues, and by GroEL (chaperonin 60). Both can protect G6PD against glycation-induced inactivation [33].

Previous methods for rectifying the loss of GSH in tissues have involved treatment with GSH, GSH esters, N-acetylcysteine or other precursors of cysteine [1,2]. The esters are used because they pass through cell membranes more easily than GSH itself.

The intracellular reducing environment modulates cytoregulation and cytotoxicity by reactive oxygen species

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Introduction

The involvement of reactive oxygen species (ROS) in cellular regulatory and cytotoxic processes implies that the cellular redox environment plays a major role in their action. The outcome depends on the concentration of ROS and the availability of reducing equivalents, both intra- and extra-cellularly. The potential toxicity of ROS has been well studied since the inception of superoxide dismutase as a probe of one-electron reduction reactions of oxygen. Although much is understood about the involvement of ROS in cytotoxic processes, several important issues require further elucidation, particularly those related to the role of the reducing environment. Recent evidence indicates that, besides their capacity to cause cytotoxicity, ROS can promote cell growth [1], stimulate protein tyrosine phosphorylation associated with growth-factor-mediated signalling [2], activate transcriptional regulators [3] and enhance the expression of proto-oncogenes [4]. In this paper we present two approaches for elucidating the role of the reducing environment in cytoregulation and cytotoxicity: an investigation of the involvement of ROS and other oxidants in growth-factor-mediated signalling and in glucose-6-phosphate dehydrogenase (G6PD) deficiency.

ROS and growth-factor-mediated signalling

Protein tyrosine phosphorylation, which is primarily triggered by association of a growth factor with its specific receptor at the cell surface, is essential to signalling. Cellular levels of tyrosine phosphorylation are tightly regulated by a family of enzymes called protein tyrosine phosphatases (PTPs) [5,6]. All PTPs have an essential cysteine residue in their catalytic domain, and total

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