Selective Modification of Glutathione Metabolism

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Summary. Glutathione, a tripeptide thiol found in virtually all cells, functions in metabolism, transport, and cellular protection. It participates in the reduction of disulfides and other molecules, and conjugates with compounds of exogenous and endogenous origin. It protects cells against the destructive effects of reactive oxygen intermediates and free radicals. Modifications of glutathione metabolism may be achieved by administration of selective enzyme inhibitors, and also by giving compounds that increase glutathione synthesis. Such effects are useful in chemotherapy and radiation therapy and in protecting cells against the toxic effects of drugs, other foreign compounds, and oxygen.

Glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH) occurs in animal cells and also in most plants and bacteria. Although this tripeptide has been known for many years, it did not become the subject of intensive study until about a decade ago when new information became available about its enzymology and metabolism. Today we have a fairly comprehensive picture of GSH metabolism and its multifunctional significance (*1*). Indeed, the diverse functions of GSH are relevant to many fields of biology, including not only enzymology and transport but also pharmacology, radiation biology, cancer therapy, toxicology, endocrinology, microbiology, and agriculture.

Glutathione functions in the reduction of the disulfide linkages of proteins and other molecules, in the synthesis of the deoxyribonucleotide precursors of DNA, and in the protection of cells against the effects of free radicals and of reactive oxygen intermediates (for example, peroxides) that are formed in metabolism. Glutathione is transported out of cells. This process seems to be connected with a transport system for γ-glutamyl amino acids, reactions that involve the cell membrane and its immediate environment, and inter-organ transport of amino acid sulfur. Glutathione has a role in the inactivation of a number of drugs and in the metabolic processing of certain endogenous compounds, such as estrogens, prostaglandins, and leukotrienes. It is also a coenzyme for several enzymes.

Our present understanding of the metabolism and physiological functions of GSH has received major impetus from studies on the enzymes involved in its metabolism, especially from the selective inhibition of these enzymes by compounds that are active in vivo. Such inhibitors, and the finding of other compounds that increase GSH synthesis, make it possible to effectively manipulate the metabolism of this compound and thereby to achieve useful effects.

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Glutathione Metabolism and Function

Recent advances in our knowledge of GSH metabolism have come from studies on (i) the reactions of the γ-glutamyl cycle, which account for the synthesis and degradation of GSH and involve transport of GSH out of cells and of γ-glutamyl amino acids into cells (*2*); (ii) the reactions involved in reversible conversion of GSH to glutathione disulfide (GSSG) (*3*); and (iii) the formation and metabolic transformations of *S*-substituted GSH conjugates (*4*). These enzymatic and transport phenomena are outlined in Fig. 1. Glutathione, synthesized intracellularly (reactions **1** and **2** in Fig. 1), is transported across cell membranes (reaction **3** in Fig. 1). Intracellular GSH is the source of plasma GSH and GSSG; both are substrates of membrane-bound γ-glutamyl transpeptidase (**4**), which transfers the γ-glutamyl moiety of GSH (and GSSG) to amino acid acceptors to form γ-glutamyl amino acids and cysteinylglycine (CysH-Gly). Transpeptidation is a major function (*2, 5*) of this enzyme, which can also hydrolyze GSH (and GSSG) to glutamate and CysH-Gly (and its disulfide). Cleavage of CysH-Gly (and its disulfide) is catalyzed by dipeptidase. The products formed by transpeptidase and dipeptidase (free amino acids, γ-glutamyl amino acids) are transported into cells (reactions **5** and **6** in Fig. 1). Transported γ-glutamyl amino acids (**6**) are substrates of γ-glutamyl cyclotransferase (**7**), which cyclizes the glutamyl moiety of γ-glutamyl amino acids to 5-oxoproline, liberating the free amino acids. 5-Oxoproline is decyclized by 5-oxoprolinase (**8**) to glutamate in a reaction coupled to cleavage of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). These reactions account for the cellular turnover of GSH.

Glutathione *S*-transferases (reaction **9** in Fig. 1) catalyze reactions between GSH and a wide variety of electrophilic compounds (X) of exogenous origin to form GSH conjugates. A GSH-reactive electrophilic moiety may be introduced into a molecule by another reaction, for example, by action of microsomal oxygenases to form an epoxide, which reacts with GSH. Compounds formed endogenously also form GSH conjugates; thus, leukotriene A, an epoxide derived from arachidonic acid, reacts with GSH to form the conjugate leukotriene C (*6*). Glutathione conjugates, like GSH, are transported across cell membranes (reaction **10** in Fig. 1); they are substrates of γ-glutamyl transpeptidase (**4**), which converts them to *S*-substituted cysteinylglycines, which are cleaved to glycine and *S*-substituted cysteines [Cys(X)]. Compounds of this type may accept the γ-glutamyl moiety of GSH to form γ-Glu-Cys(X) (*7–9*), which may be transport forms of Cys(X) (reaction **11** in Fig. 1) (in analogy with other γ-glutamyl amino acids). Acetylation of Cys(X) occurs (reaction **12**) to form *N*-acetyl *S*-substituted cysteines (mercapturic acids), which are transported (reaction **13** in Fig. 1) and ultimately excreted in the urine or feces.

The pathways described above, which include intracellular and extracellular reactions and transport phenomena, are involved in amino acid transport (principally cystine and glutamine), detoxication of drugs and other foreign compounds, and processing of certain endogenous metabolites. The transport of GSH (reaction **3** in Fig. 1) provides substrate for γ-glutamyl transpeptidase (reaction **4** in Fig. 1) and thus for formation of γ-glutamyl amino acids; GSH transport to the blood plasma functions in the

inter-organ translocation of cysteine sulfur (*2, 10*). The generality of GSH transport suggests that this process may have a role in the protection of cell membranes. Since there is no extracellular mechanism for reduction of GSSG, GSH must be continually supplied from the cell (*11*).

In contrast to the pathways of metabolism in which GSH undergoes turnover and conjugation, those in which the tripeptide is reversibly converted to GSSG take place exclusively within cells. Glutathione participates in thiol-disulfide exchange reactions (reaction **14** in Fig. 1) with low molecular weight disulfides and with protein disulfide bonds. The latter are involved in synthesis and breakdown of protein molecules and in the regulation of certain enzymes. In one significant pathway for the reduction of ribonucleoside diphosphates to the corresponding 2′-deoxyribose compounds, the protein glutaredoxin is reduced by GSH (*12*).

Glutathione is of major importance in the reduction of hydrogen peroxide and organic peroxides (for example, lipid peroxides) (reaction **15** in Fig. 1), reactions that are catalyzed by selenium-containing GSH peroxidase and by other proteins that also exhibit GSH *S*-transferase activity. Both GSH peroxidase and catalase promote removal of hydrogen peroxide, a dismutation product of superoxide radicals (*13*). Through this system and by other mechanisms, GSH participates in the destruction of free radicals (reaction **16** in Fig. 1). Peroxides (*14*) and free radicals (*15*) are formed in normal metabolism and have physiological functions (for example, in phagocytosis); they are produced in increased amounts after irradiation, after the administration of certain drugs, and in the presence of increased oxygen tension. The high ratio (about 100:1) of GSH to GSSG found intracellularly is maintained by the activity of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent GSSG reductase (reaction **17** in Fig. 1), a widely distributed flavoprotein. NADPH is formed in the erythrocyte through the actions of glucose 6-phosphate and 6-phosphogluconate dehydrogenases; these and other dehydrogenases produce NADPH in other tissues.

Consequences of Abnormalities of GSH Metabolism in Man

Significant clues to the functions of GSH have come from studies of patients with deficiencies of specific enzymes of GSH metabolism (*16*). Patients with deficiency of GSH synthetase (reaction **2** in Fig. 1) have decreased tissue concentrations of GSH, accumulate 5-oxoproline in their blood plasma and cerebrospinal fluid, and excrete massive amounts of this compound in their urine. Their symptoms include defective brain function, marked acidosis, and a tendency to hemolysis. Glutathione regulates its own synthesis by feedback inhibition of γ-glutamylcysteine synthetase (*17*) (reaction **1** in Fig. 1); therefore, when there is severe GSH deficiency, γ-glutamylcysteine is overproduced and converted to 5-oxoproline by γ-glutamyl cyclotransferase (reaction **7** in Fig. 1). The formation of 5-oxoproline exceeds the capacity of 5-oxoprolinase (reaction **8** in Fig. 1), so that this compound accumulates, leading to acidosis (*18*). Acidosis is thus a secondary effect of GSH deficiency. Brain dysfunction occurs in patients whose acidosis has been controlled since birth. The brain defects seem therefore to be directly associated with GSH deficiency, perhaps being due to lack of its protective functions or to a role played by the tripeptide in neurotransmission. Patients with γ-glutamylcysteine synthetase deficiency also exhibit increased hemolysis and brain dysfunction as well as peripheral neuropathy, myopathy, and aminoaciduria (*19*). The finding of hemolysis in patients with GSH deficiency emphasizes the protective role of the compound in the erythrocyte; patients with glucose-6-phosphate dehydrogenase deficiency, who have decreased ability to form NADPH for GSSG reduction (*20*), experience hemolysis, as do patients with GSSG reductase deficiency (*21*). Studies of two patients with deficiency of γ-glutamyl transpeptidase, a very rare condition, revealed some evidence of brain dysfunction (*22*). These patients accumulate GSH in their blood plasma and excrete in their urine large amounts of GSH, γ-glutamylcysteine, and cysteine moieties (*23*).

Selective Inhibition of Enzymes of GSH Metabolism

Studies of the enzymology of GSH metabolism have been facilitated by the design of a series of selective enzyme inhibitors. For example, inhibition of 5-oxoprolinase in mice by administration of the competitive 5-oxoprolinase inhibitor 2-imidazolidone-4-carboxylate leads to accumulation and excretion of oxoproline (*24*); administration of β-aminoglutaryl-α-aminobutyrate, a competitive inhibitor of γ-glutamyl cyclotransferase, results in decreased formation of 5-oxoproline (*25*). This indicates that 5-oxoprolinase and γ-glutamyl cyclotransferase are, respectively, major catalysts for the utilization and formation of 5-oxo-

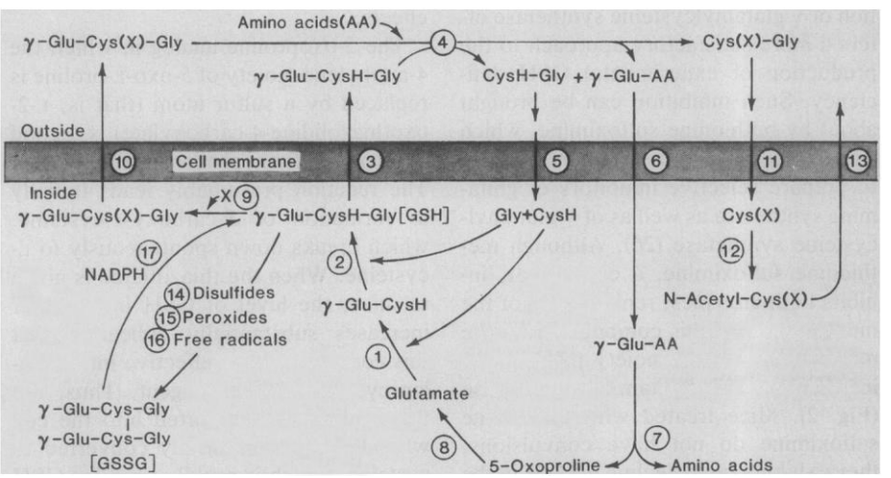

Fig. 1. Outline of GSH metabolism. The cellular turnover of GSH involves its intracellular synthesis from glutamate, cysteine, and glycine catalyzed by γ-glutamylcysteine synthetase (**1**) and GSH synthetase (**2**), followed by transport of GSH (**3**) and its conversion by membrane-bound γ-glutamyl transpeptidase (**4**) to cysteinylglycine (*CysH-Gly*) and γ-glutamyl amino acids. Cleavage of cysteinylglycine to cysteine (*CysH*) and glycine (*Gly*) may be catalyzed by membrane-bound dipeptidase (followed by transport of the free amino acids) or may occur intracellularly after transport of the dipeptide (**5**). Transported γ-glutamyl amino acids (**6**) are converted by γ-glutamyl cyclotransferase (**7**) to amino acids and 5-oxoproline; the latter is decyclized by 5-oxoprolinase (**8**) to glutamate. Glutathione reacts intracellularly with a variety of compounds of exogenous and endogenous origin (*X*) in reactions catalyzed by GSH *S*-transferases (**9**) to form GSH *S*-conjugates. These are transported (**10**) and follow pathways similar to those involved in GSH turnover (**4**, **6**, and **11**). *S*-Substituted derivatives of cysteine are acetylated (**12**) to form mercapturic acids, which are transported out of cells (**13**). Intracellular GSH is converted to GSSG in transhydrogenation reactions (**14**), in reactions catalyzed by GSH peroxidases (**15**), and by reaction with free radicals (**16**). GSSG is converted to GSH by GSSG reductase (**17**).

proline in vivo. Mice treated with inhibitors of γ-glutamyl transpeptidase have increased concentrations of GSH in their plasma and excrete in their urine substantial amounts of GSH, γ-glutamylcysteine, and cysteine moieties (*23*), findings that are virtually the same as found in patients deficient in this enzyme. Studies with transpeptidase inhibitors were of importance in showing that GSH is transported across cell membranes (*10*) and that γ-glutamyl transpeptidase catalyzes γ-glutamylcysteine formation in vivo (*8*).

Studies on the effects of GSH depletion might be expected to reveal significant information about GSH function. When GSH is depleted by the use of oxidizing agents, however, the studies may be complicated by the oxidation of other cellular components, formation of high levels of GSSG, and rapid reversibility due to GSSG reduction. Depletion of GSH by the use of compounds that react directly with GSH (for example, diethylmaleate and 1-chloro-2,4-dinitrobenzene) may be accompanied by toxicity due to nonspecific interactions; furthermore, the sudden depression of GSH concentrations may be followed by rapid resynthesis and restoration of normal concentrations.

On the basis of studies of patients with deficiency of GSH synthetase, a model for GSH depletion induced by inhibition of this enzyme would be expected to be complicated by marked acidosis. Inhibition of γ-glutamylcysteine synthetase offers a more satisfactory approach to the production of experimental GSH deficiency. Such inhibition can be brought about by buthionine sulfoximine, which was synthesized in the course of efforts to prepare selective inhibitors of glutamine synthetase as well as of γ-glutamylcysteine synthetase (*26*). Although methionine sulfoximine, a convulsant, inhibits both enzymes, replacement of the methyl group of this compound with the more bulky *n*-butyl moiety prevents its interaction with glutamine synthetase (Fig. 2). Mice treated with buthionine sulfoximine do not have convulsions; they exhibit a rapid decline in GSH in the kidney, liver, plasma, pancreas, and muscle, and after prolonged treatment also show lowered concentrations of GSH in other tissues (*10*). The rate of decline of GSH in tissues reflects its rate of utilization, which is about equivalent to its rate of transport from the cells; the rapid and marked decrease in plasma GSH concentrations reflects the substantial inter-organ transport of the tripeptide. Concentrations of GSH in various cells grown in tissue culture (*27*) and in erythrocytes in suspension (*28*) also decline rapidly in the presence of buthionine sulfoximine. Cells that have high rates of GSH turnover may be rapidly depleted of the tripeptide by inhibition of GSH synthesis. Cells with very low rates of GSH turnover might be depleted by treatment first with a compound that reacts with GSH to form a conjugate and then with an inhibitor of GSH synthesis. Although buthionine sulfoximine has been given to mice for as long as 14 days without apparent toxicity, it appears to decrease the viability of human lymphoid cells grown in vitro.

Methods of Increasing GSH Synthesis

The first step in GSH synthesis (reaction **1** in Fig. 1) is controlled by feedback inhibition by GSH, a mechanism that seems to normally determine the upper concentration of cellular GSH (*17*). Concentrations of GSH are also dependent on the supply of cysteine, which is derived from dietary protein and by transsulfuration from methionine in the liver. Synthesis of GSH can be increased, under certain conditions, by increasing the supply of substrates to the two synthetases (reactions **1** and **2** in Fig. 1). Administration of cysteine is not an ideal way to increase GSH concentrations because this amino acid is rapidly metabolized; furthermore, it is toxic (*29*), apparently, in part, because of its extracellular effects.

The 5-oxoproline analog in which the 4-methylene moiety of 5-oxo-L-proline is replaced by a sulfur atom (that is, L-2-oxothiazolidine-4-carboxylate) is a good substrate of 5-oxoprolinase (Fig. 3) (*30*). The reaction presumably leads initially to formation of *S*-carboxy-L-cysteine, which breaks down spontaneously to L-cysteine. When the thiazolidine is given to mice, the level of GSH in the liver increases substantially indicating that this compound is an effective intracellular cysteine delivery agent. Thus, the thiazolidine is transported into the cell where it is enzymatically converted to cysteine, which is rapidly used for GSH synthesis. This interpretation is supported by the finding that administration of buthionine sulfoximine prevents the increase in liver GSH found after giving the thiazolidine.

Another way in which tissue GSH concentrations may be increased is by administration of γ-glutamylcysteine (or its disulfide form) or of γ-glutamylcystine (*31*). Studies in which the model compound γ-glutamylmethionine sulfone was given to mice showed that the kidney has a transport system for γ-glutamyl amino acids that is not shared by the corresponding free amino acids (*32*). Administration to mice of glutamate and cysteine or cystinyl-*bis*-glycine leads to only a small increase in the GSH level of the kidney. However, when γ-glutamylcysteine (or related compounds) are given, high GSH levels are obtained because the feedback-regulated step is bypassed; the administered γ-glutamyl amino acid is transported intact and serves as substrate of GSH synthetase. These findings suggest that there may be an alternative pathway of GSH synthesis in some cells in which γ-glutamylcyst(e)ine, formed by transpeptidation, is transported and utilized (*31*).

Increased tissue concentrations of GSH have been found after administration of butylated hydroxyanisole and similar compounds (*33*); as discussed below, increased tissue levels of GSH may be associated with administration of certain drugs and carcinogens, increased oxygen tension, and hyperthermia. The mechanisms responsible for increasing GSH levels require study.

Useful Effects of Modification of GSH Metabolism

The possibility that depletion of cellular GSH by treatment with sulfoximine inhibitors of γ-glutamylcysteine synthetase might make tumor cells more susceptible to irradiation and certain chemotherapeutic agents (*34*) is now being examined. In this approach the destructive effects of reactive oxygen intermediates (such as superoxide and peroxide) and free radicals are used advantageously. Glutathione depletion may be useful in chemotherapeutic situations in which the cells to be killed and the cells to be spared have substantially different quantitative requirements for GSH. Many normal cells probably have a considerable excess of GSH. Certain tumors and parasites, in contrast, may have GSH concentrations that are close to the minimum required for cell survival. It has long been known that irradiation leads to a decrease in cellular thiols and that thiols protect cells against the effects of irradiation. Tumors that are relatively resistant to irradiation and have high GSH concentrations would be expected to become more radiosensitive after treatment with an inhibitor of GSH synthesis.

In studies on several human lymphoid lines, cells depleted of GSH (to 5 percent of the control levels) by incubation in media containing buthionine sulfoximine

were much more sensitive to γ-irradiation than the controls, as indicated by their decreased viability (measured by trypan blue exclusion) (*27*). In experiments on cultured human lung carcinoma cells in which viability was determined by clonogenic survival, buthionine sulfoximine–induced depletion of GSH was also found to increase sensitivity to irradiation (*35*). In studies on V79 cells, GSH depletion produced by buthionine sulfoximine led to selective sensitization to irradiation under hypoxic conditions and to a decrease of the oxygen enhancement ratio (*36*). Selective sensitization of hypoxic cells is of importance in radiation therapy of tumors because hypoxic cells are much less sensitive to radiation than are oxic cells (*37*).

Studies on the oxidative cytolysis of several tumor cell lines by glucose oxidase, and by activated macrophages and granulocytes in the presence of phorbol myristate acetate, showed that depletion of GSH by incubation in media containing buthionine sulfoximine enhanced cytolysis. Recovery of tumor cell resistance to peroxide was closely correlated with resynthesis of cellular GSH (*38*).

The protozoan parasite *Trypanosoma brucei brucei*, which contains no catalase, has a very high intracellular concentration of hydrogen peroxide, about 70 μ*M*, which is at least 100 times higher than the concentration in mammalian cells. Depletion of GSH would therefore be expected to be more damaging to the parasite than to the host. When six mice infected with this parasite were treated with buthionine sulfoximine, two of them were cured and four survived significantly longer than untreated control mice (*39*). The sensitivity of the malarial parasite (*Plasmodium* species) to oxidant stress (*40*) suggests that therapy leading to decreased GSH synthesis might also be useful in treating infections with this protozoan parasite.

Depletion of GSH by inhibition of its synthesis may serve as a valuable adjuvant in chemotherapy with drugs that are detoxified by reactions involving GSH. Development of resistance to a drug or to radiation may be associated with an increase in cellular GSH. For example, murine L1210 leukemia cells resistant to therapy with L-phenylalanine mustard may have GSH levels that are about twice those of sensitive L1210 cells (*41*). L-Phenylalanine mustard is detoxified by conversion to a nontoxic derivative in a GSH-dependent dechlorination reaction. Treatment of resistant cells in vitro with buthionine sulfoximine led to sensitization of the cells to the agent, and when tumor-bearing mice were sensitized to the effects of L-phenylalanine mustard by continuous intraperitoneal infusion of buthionine sulfoximine there was an increase in the life-span of these animals.

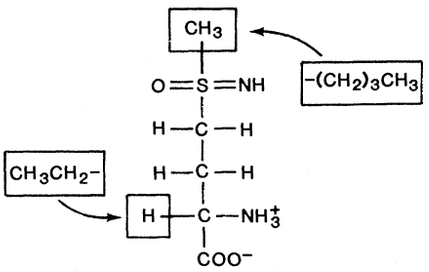

Fig. 2. Structure of methionine sulfoximine. This compound inhibits both the synthesis of GSH and of glutamine. Replacement of the methyl group of methionine sulfoximine by an *n*-butyl group gives buthionine sulfoximine, which is a selective inhibitor of γ-glutamylcysteine synthetase (reaction **1** in Fig. 1) and therefore of GSH synthesis. Substituting an α-ethyl group for the α-hydrogen atom gives a selective inhibitor of glutamine synthetase (*26*).

The inhibition of GSH synthesis may also prove useful in treating infections due to antibiotic-resistant microorganisms (*42*) and in preventing the induction of thermotolerance, a phenomenon associated with increased GSH (*43*). Certain other effects observed in experimental systems are also of interest; for example, GSH depletion by treatment with buthionine sulfoximine led to decreased synthesis of prostaglandin E_2 and leukotriene C by macrophages (*44*). Conversion of leukotriene C to leukotriene D, which is catalyzed by γ-glutamyl transpeptidase (*7*), could probably be inhibited in vivo by administration of an inhibitor of this enzyme. Substantial inhibition of the transpeptidase can be achieved in vivo (*2, 10, 23*). Glutathione is required for lectin-induced lymphocyte activation, which is inhibited by treatment of the cells with buthionine sulfoximine (*45*).

Although useful effects may be achieved by depletion of GSH, the normal physiological functions of GSH in protecting against reactive oxygen intermediates, free radicals, and toxic compounds suggest that an increase in cellular GSH may also be beneficial under certain conditions. As discussed above, GSH concentrations can be increased by supplying extra substrate to the two synthetases (reactions **1** and **2** in Fig. 1).

Since GSH serves effectively in the detoxication of many drugs, the GSH status of an animal is of importance in protection against toxicity (*46*). This has been examined extensively in the case of acetaminophen, which is metabolized to a highly reactive intermediate that interacts effectively with various cell constituents (*47*). A significant pathway of acetaminophen detoxication involves conjugation with GSH, and acetaminophen toxicity is associated with marked decrease of hepatic GSH concentrations. Administration of L-2-oxothiazolidine-4-carboxylate protects against acetaminophen toxicity by promoting GSH synthesis. *N*-Acetyl-L-cysteine also promotes GSH synthesis, but less effectively than the thiazolidine (*30*). The thiazolidine (and other compounds that increase cysteine formation) would probably also protect against other toxic agents. Tumor cells that are deficient in 5-oxoprolinase, in contrast to normal cells, would not be protected against such toxic agents by the thiazolidine.

The thiazolidine may have addditional usefulness as a component of amino acid mixtures used in diets and in solutions used for parenteral administration. The available preparations usually do not contain cysteine because of its toxicity and its rapid conversion to the very insoluble amino acid cystine. Treatment with the thiazolidine may be of value to patients with liver disease (*48*) and to premature infants (*49*), who may be deficient in the utilization of methionine sulfur for cysteine formation, and thus in GSH synthesis. Such an approach may also be of value in protecting against oxygen toxicity; increased synthesis of lung GSH occurs in response to expo-

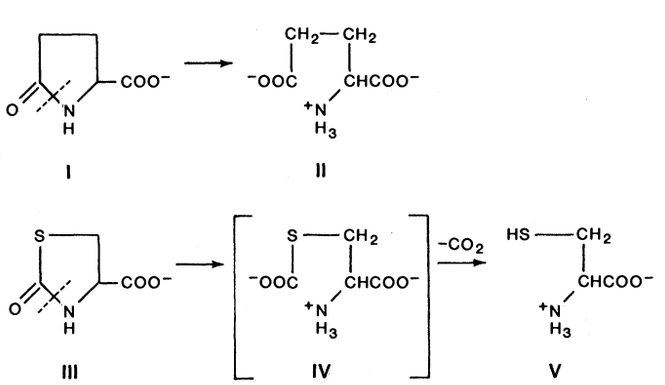

Fig. 3. Reactions catalyzed by 5-oxoprolinase. 5-Oxoproline (**I**) is converted to glutamate (**II**), and 2-oxothiazolidine-4-carboxylate (**III**) is converted to cysteine (**V**). Both reactions are coupled to cleavage of adenosine triphosphate to adenosine diphosphate and inorganic phosphate.

sure to increased oxygen tension, and animals deficient in cysteine (and therefore GSH) cannot survive in atmospheres containing high concentrations of oxygen (*50*). The thiazolidine may also be a useful substitute for cysteine in tissue culture media, especially for growth of cells that are sensitive to the toxic effects of cysteine (*51*).

The effectiveness of the thiazolidine as an intracellular cysteine precursor depends on the presence of 5-oxoprolinase, an enzyme activity found in almost all animal cells. Its occurrence also in plants (*52*) suggests that the thiazolidine may be useful as a safener in agriculture to protect crop plants against the toxic effects of herbicides. Strategies involving the combined use of toxic agents and the thiazolidine might be effective, especially when favorable quantitative differences exist between the 5-oxoprolinase activities of the weeds and the crop plants. Thus, a relatively low level of 5-oxoprolinase in the weeds might facilitate selective protection of the crop plant. The relative activities of the GSH *S*-transferases might also be usefully exploited; thus, a plant that lacks this activity would be expected to be more susceptible to the effects of certain herbicides.

Discussion

The currently available information about GSH metabolism and its selective modification by enzyme inhibitors and by compounds that lead to increased GSH synthesis has led to some promising results. Glutathione depletion has potential value in chemotherapy and radiation therapy. Procedures that increase GSH concentrations are valuable in protecting against certain toxicities, including that due to oxygen. Only some of the potentialities of modifying GSH metabolism have thus far been explored. Tissue selective approaches seem feasible; for example, the kidney transport system for γ-glutamyl amino acids might be exploited. That treatment with γ-glutamylcysteine and similar compounds increases concentrations of GSH in kidney (*31*) suggests a useful method for selective protection of this organ.

Concentrations of GSH in tissues decline with age (*53*), a phenomenon not yet fully explained. The long-term effects of moderate GSH depletion on cellular function and mutagenesis need to be studied. It has been reported that the mean life-span of mice can be extended by dietary antioxidants (*54*). There are apparent connections between GSH metabolism and carcinogenesis (*34*). Thus, certain carcinogens increase GSH levels and the activities of γ-glutamyl transpeptidase and GSH *S*-transferases. The reactive forms of carcinogens are electrophiles which may conjugate with GSH. This is currently a very active area of research (*4*). Oral administration of GSH to rats bearing aflatoxin-induced liver tumors was reported to lead to tumor regression (*55*). This finding, which requires confirmation (and if confirmed, explanation), might conceivably be related to stimulation by GSH, under aerobic conditions, of lipid peroxidation; although GSH normally functions in vivo to protect cell membranes, its extracellular oxidation under certain conditions in vitro has been shown to induce lipid peroxidation (*56*).

The recent work on GSH metabolism and its modification must be considered in relation to several closely associated phenomena. Decreased GSH synthesis, which leads to decreased function of GSH peroxidase, may not necessarily affect normal cells, which have superoxide dismutase and catalase activities; some tumor cells have diminished levels of these activities (*57*) and may, therefore, be damaged. The destruction of reactive oxygen intermediates and of free radicals involves the activities of superoxide dismutase, catalase, GSH peroxidase, and GSSG reductase as well as GSH and a supply of NADPH. This suggests the potential usefulness of combined therapies involving selective modification of GSH metabolism and inhibition of other components of this complex system.

The current interest in GSH will probably lead to new findings about its metabolism and functions, and thus indicate additional useful metabolic manipulations. Further progress in the development of more potent selective inhibitors, and other compounds that stimulate GSH synthesis, should improve the effectiveness of present approaches and facilitate their extension to other biological problems.

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Mathematics and Science Learning: A New Conception

Lauren B. Resnick

In the last few years a new consensus on the nature of learning has begun to emerge, stimulated by research in the field that has come to be known as cognitive science. The emerging conception of learning has a direct bearing on how science and mathematics can be taught most effectively.

Summary. Findings in cognitive science suggest new approaches to teaching in science and mathematics.

I will sketch here a few examples of recent findings in cognitive science, many of which support the intuition of our most thoughtful teachers. In physics and other sciences, according to these studies, even students who do well on textbook problems often cannot apply the laws and formulas they have been drilled on to interpreting actual physical events. This observation has been made on all kinds of students, including gifted middle-school children and students at some of our most prestigious universities (*1, 2*). The inability to apply routines learned in school is consistent with recent findings from the National Assessment of Educational Progress showing that mathematical problem-solving skills of American children lag far behind their calculation abilities (*3*).

Another well-supported finding is that all students, the weak as well as the strong learners, come to their first science classes with surprisingly extensive theories about how the natural world works. They use these "naïve" theories to explain real world events before they have had any science instruction. Then, even after instruction in new concepts and scientifically supported theories, they still resort to their prior theories to solve any problems that vary from their textbook examples (*4–6*). Some studies have shown that students' prior theories can actually interfere with learning scientific concepts. The students' naïve theories affect what they perceive to be happening in classroom demonstrations or laboratory experiments, and they continue to attach their naïve meanings to technical terms (for example, the term acceleration).

Several studies show that successful problem-solving requires a substantial amount of qualitative reasoning (*7–9*). Good problem-solvers do not rush in to apply a formula or an equation. Instead, they try to understand the problem situation; they consider alternative representations and relations among the variables. Only when they are satisfied that they understand the situation and all the variables in it in a qualitative way do they start to apply the quantification that we often mistakenly identify as the essence of "real" science or mathematics.

These demonstrations of the potent role of naïve theories in science learning, and of the central role of qualitative understanding of a situation in problem-solving, contribute to a new conception of the learner and the learning process that is emerging from cognitive research in mathematics and science. This research has in just a few years produced a new consensus on the nature of learning that is not yet widely reflected in the way mathematics and science teaching is conducted in the schools.

There are many complexities, but the fundamental view of the learner that is emerging can be expressed quite simply.

First, learners construct understanding. They do not simply mirror what they are told or what they read (*10, 11*). Learners look for meaning and will try to find regularity and order in the events of the world, even in the absence of complete information. This means that naïve theories will always be constructed as part of the learning process.

Second, to understand something is to know relationships. Human knowledge

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