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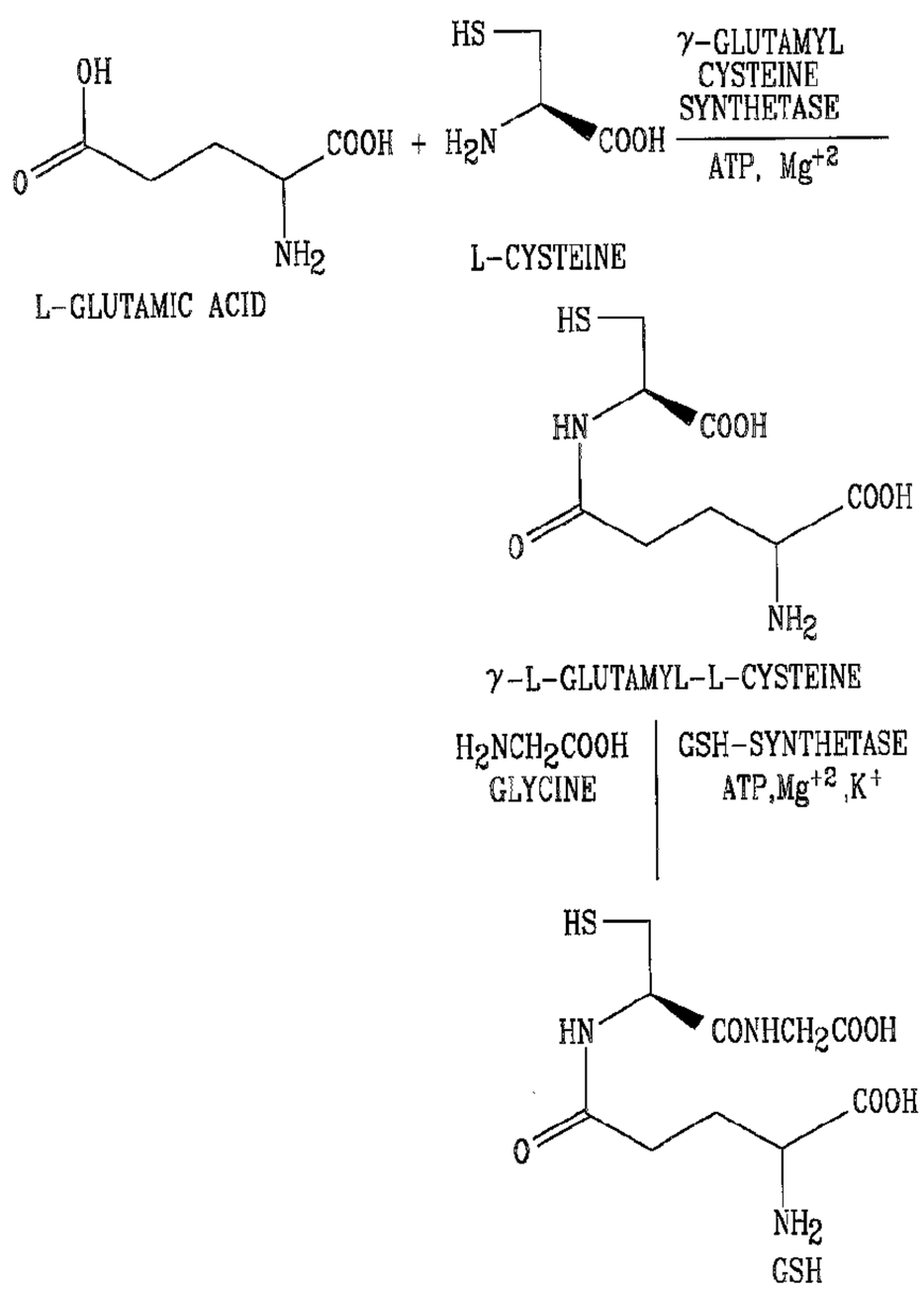
(54) Titre : UTILISATION DE RIBOSE-CYSTEINE POUR LE TRAITEMENT DE L'HYPOXIE PAR ADMINISTRATION ACCRUE DE GLUTATHIONE ET DES NIVEAUX D'ATP DANS LES CELLULES

(54) Title: USE OF RIBOSE-CYSTEINE TO TREAT HYPOXIA BY ENHANCING DELIVERY OF GLUTATHIONE AND ATP LEVELS IN CELLS



(57) Abrégé/Abstract:
A therapeutic method is provided comprising treating a mammal subject to hypoxia with an amount of 2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)-carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof effective to both maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in said tissue.

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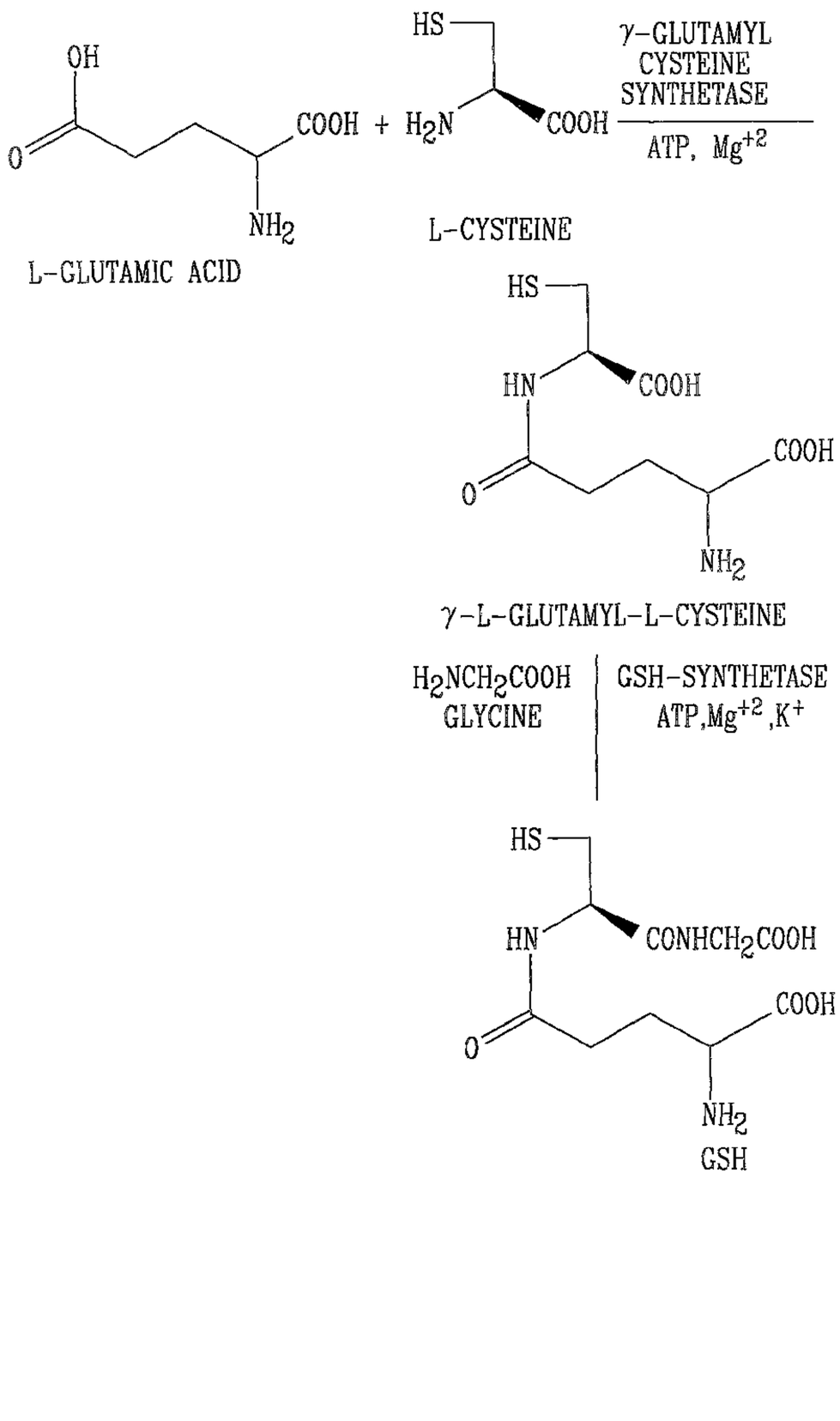

(57) Abstract: A therapeutic method is provided comprising treating a mammal subject to hypoxia with an amount of 2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)-carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof effective to both maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in said tissue.

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USE OF RIBOSE-CYSTEINE TO TREAT HYPOXIA BY ENHANCING DELIVERY OF GLUTATHIONE AND ATP LEVELS IN CELLS

BACKGROUND OF THE INVENTION

The protective mechanisms of mammalian cells against exogeneous and endogenous stressors that generate harmful free radicals employ the antioxidant co-enzyme, glutathione (GSH). GSH is important in maintaining the structural integrity of cell and organelle membranes and in the synthesis of microtubules and macromolecules. See C.D. Klassen et al. Fundamental and Applied Toxicology, 5, 806 (1985). Stimulation of GSH synthesis in rat renal epithelial cells and stomach cells has been found to protect the cells from the toxic effects of cyclophosphamide and serotonin, respectively. Conversely, inhibition of glutathione synthesis and glutathione depletion has been found to have the following effects: (a) decreased cell viability, (b) increased sensitivity of cells to the effects or irradiation, (c) increased sensitivity of tumor cells to peroxide cytolysis, (d) decreased synthesis of prostaglandin E and leukotriene C and (e) selective destruction of trypanosomes in mice.

Biosynthesis of glutathione (GSH) involves two sequential reactions that utilize ATP and that are catalyzed by the enzymes γ-glutamylcysteine synthetase and glutathione synthetase (GSH-synthetase) using the three precursor amino acids L-glutamic acid, L-cysteine, and glycine, as shown in Fig. 1.

All substrate-level reactants occur at near enzyme-saturating concentrations *in vivo* with the exception of L-cysteine, whose cellular concentration is exceedingly low. Therefore, the first reaction in which L-cysteine is required, i.e., the synthesis of γ-L-glutamyl-L-cysteine, is the rate-limiting step of glutathione biosynthesis. Thus, the availability of intracellular L-cysteine is a critical factor in the overall biosynthesis of GSH, are sufficient stores of ATP.

In the synthesis of ATP via the nucleotide salvage pathway, the nucleotide precursors that may be present in the tissue are converted to AMP and further phosphorylated to ATP. Adenosine is directly phosphorylated to AMP,

while xanthine and inosine are first ribosylated by 5-phosphoribosyl-1-pyrophosphate (PRPP) and then converted to AMP.

Ribose is found in the normal diet only in very low amounts, and is synthesized within the body by the pentose phosphate pathway. In the de novo synthetic pathway, ribose is phosphorylated to PRPP, and condensed with adenine to form the intermediate adenosine monophosphate (AMP). AMP is further phosphorylated via high energy bonds to form adenosine diphosphate (ADP) and ATP.

During energy consumption, ATP loses one high energy bond to form ADP, which can be hydrolyzed to AMP. AMP and its metabolites adenine, inosine and hypoxanthine are freely diffusible from the muscle cell and may not be available for resynthesis to ATP via the salvage pathway.

The availability of PRPP appears to control the activity of both the salvage and de novo pathways, as well as the direct conversion of adenine to ATP. Production of PRPP from glucose via the pentose phosphate pathway appears to be limited by the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Glucose is converted by enzymes such as G6PDH to ribose-5-phosphate and further phosphorylated to PRPP, which augments the de novo and salvage pathways, as well as the utilization of adenine.

Many conditions produce hypoxia. Such conditions include acute or chronic ischemia when blood flow to the tissue is reduced due to coronary artery disease or peripheral vascular disease where the artery is partially blocked by atherosclerotic plaques. In U.S. Pat. No. 4,719,201, it is disclosed that when ATP is hydrolyzed to AMP in cardiac muscle during ischemia, the AMP is further metabolized to adenosine, inosine and hypoxanthine, which are lost from the cell upon reperfusion. In the absence of AMP, rephosphorylation to ADP and ATP cannot take place. Since the precursors were washed from the cell, the nucleotide salvage pathway is not available to replenish ATP levels. It is disclosed that when ribose is administered via intravenous perfusion into a heart recovering from ischemia, recovery of ATP levels is enhanced.

Transient hypoxia frequency occurs in individuals undergoing anesthesia and/or surgical procedures in which blood flow to a tissue is temporarily interrupted. Peripheral vascular disease can be mimicked in intermittent claudication where temporary arterial spasm causes similar symptoms. Finally,

persons undergoing intense physical exercise or encountering high altitudes may become hypoxic. U.S. Pat. No. 6,218,366 discloses that tolerance to hypoxia can be increased by the administration of ribose prior to the hypoxic event.

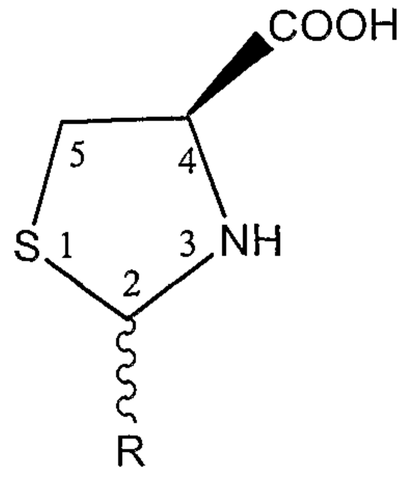
Hypoxia or ischemia can also deplete GSH. For example, strenuous aerobic exercise can also deplete antioxidants from the skeletal muscles, and sometimes also from the other organs. Exercise increases the body's oxidative burden by calling on the tissues to generate more energy. Making more ATP requires 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 effective in maintaining performance levels. See L.L. Ji, Free Rad. Biol. Med., 18, 1079 (1995).

Tissue injury, as from burns, ischemia and reperfusion, surgery, septic shock, or trauma can also deplete tissue GSH. See, e.g., K. Yagi, Lipid Peroxides in Biology and Medicine, Academic Press, N.Y. (1982) at pages 223-242; A. Blaustein et al., Circulation, 80, 1449 (1989); H.B. Demopoulos, Pathology of Oxygen, A.P. Autor, ed., Academic Press, N.Y. (1982) at pages 127-128; J. Vina et al., Brit. J. Nutr., 68, 421 (1992); C.D. Spies et al., Crit. Care Med., 22, 1738 (1994); B.M. Lomaestro et al., Annals. Pharmacother., 29, 1263 (1995) and P.M. Kidd, Alt. Med. Res., 2, 155 (1992).

It has been hypothesized that delivery of L-cysteine to mammalian cells can elevate GSH levels by supplying this biochemical GSH precursor to the cell. However, cysteine itself is neurotoxic when administered to mammals, and is rapidly degraded. In previous studies, it was shown that N-acetyl-L-cysteine, L-2-oxothiazolidine-4-carboxylate, as well as 2(R,S)-n-propyl-, 2(R,S)-n-pentyl and 2(R,S)-methyl-thiazolidine-4R-carboxylate can protect mice from heptatotoxic dosages of acetaminophen. See H.T. Nagasawa et al., J. Med. Chem., 27, 591 (1984) and A. Meister et al., U.S. Pat. No. 4,335,210. L-2-Oxothiazolidine-4-carboxylate is converted to L-cysteine via the enzyme 5-oxo-L-prolinase. As depicted in Fig. 2, compounds of formula 1, e.g., wherein R=CH_3, function as prodrug forms of L-cysteine (2), liberating this sulfhydryl amino aciuc by nonenzymatic ring opening and hydrolysis. However, the dissociation to yield L-cysteine necessarily releases an equimolar amount of the

aldehyde (3), RCHO. In prodrugs in which R is an aromatic or an alkyl residue, the potential for toxic effects is present.

U.S. Pat. No. 4,868,114 discloses a method comprising stimulating the biosynthesis of glutathione in mammalian cells by contacting the cells with an effective amount of a compound of the formula (1):

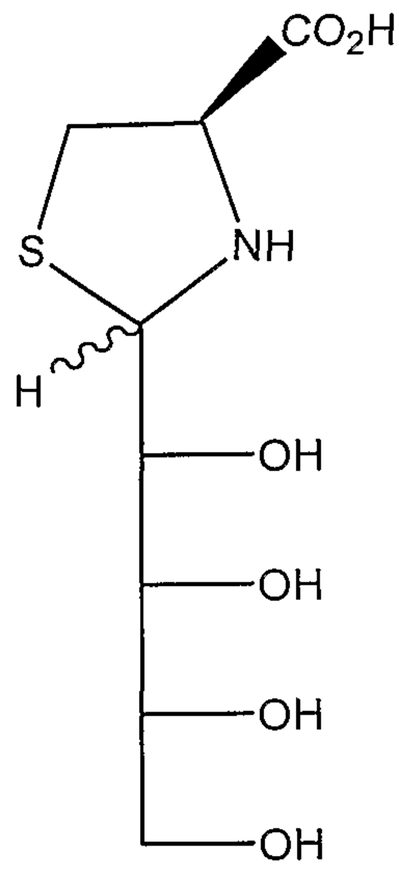


(1)

wherein R is a $(CHOH)_nCH_2OH$ and wherein n is 1-5. The compound wherein n is 3 is 2(R,S)-D-ribo-(1′, 2′, 3′, 4′-tetrahydroxybutyl)thiazolidone-4(R)-carboxylic acid (Ribose-Cysteine, RibCys). Following *in vivo* administration, RibCys releases cysteine by non-enzymatic hydrolysis. RibCys has been demonstrated to be effective to protect against acetaminophen-induced hepatic and renal toxicity. A.M. Lucus, Toxicol. Pathol., 28, 697 (2000). RibCys can also protect the large and small bowel against radiation injury. See M.P. Caroll et al., Dis. Colon Rectum, 38, 716 (1995). These protective effects are believed to be due to the stimulation of GSH biosynthesis, which elevates intracellular GSH. However, a need exists for methods to restore or maintain intracellular GSH stores in mammalian tissues subjected to hypoxic conditions in which the ATP stores necessary to drive the biosynthesis of GSH and its precursors are depleted.

SUMMARY OF THE INVENTION

The present invention provides a method to treat a mammal threatened by, or afflicted with a hypoxic condition (hypoxia) comprising administering an effective amount of a compound of formula (Ia):



(Ia)

(RibCys) or a pharmaceutically acceptable salt thereof, effective to counteract the effects of said hypoxia in the tissue(s) of said mammal. The present invention also provides the use of a compound of formula I(a) or a salt thereof to prepare a medicament useful to treat a mammal, such as a human, threatened by or afflicted with a hypoxic condition (hypoxia).

Although depressed glutathione levels have been implicated in a number of hypoxic conditions, as discussed above, the use of RibCys or its salts to prevent, counteract or otherwise treat such conditions has not been reported. It is believed that simply administering a GSH precursor such as cysteine will not be as effective in many instances of hypoxia, when the depletion of ATP stores contributes to inhibition to the biosynthesis of GSH. As well as functioning as a prodrug for cysteine, administration of effective amounts of RibCys can deliver amounts of ribose to ATP-depleted tissues that stimulate the *in vivo* synthesis of ATP and that also can stimulate the synthesis of NADPH (nicotinamide adenine dinucleotide phosphate, reduced). This coenzyme supplies the electrons to glutathione reductase, which in turn recycles oxidized GSH via GSSG, to free GSH, which resumes its protective role as a cofactor for antioxidant enzymes in the cell. Optionally, compound (Ia) can be administered with an additional amount of free ribose. Optionally, a medicament containing compound I(a) can

contain an additional amount of free ribose. Preferably, administration will be by oral administration, particularly in prophylactic or pre-loading situations, but parenteral administration, as by injection or infusion, may be necessary in some situations.

In accordance with one aspect of the present invention, there is provided use of RibCys or a pharmaceutically acceptable salt thereof in the preparation of a medicament to effectively maintain, restore or increase both the ATP levels and the glutathione levels so as to treat hypoxia in a mammal threatened with, or afflicted with, a hypoxic condition.

In accordance with a further aspect of the present invention, there is provided use of RibCys or a pharmaceutically acceptable salt thereof to effectively maintain, restore or increase both the ATP levels and the glutathione levels so as to treat hypoxia in a mammal threatened with, or afflicted with, a hypoxic condition.

In accordance with a further aspect of the present invention, there is provided use of an amount of 2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof in the preparation of a medicament to effectively maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in a tissue in a mammal subject to hypoxia.

In accordance with a further aspect of the present invention, there is provided use of an amount of 2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof to effectively maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in a tissue in a mammal subject to hypoxia.

In accordance with a further aspect of the present invention, there is provided use of RibCys in the preparation of a medicament in an effective amount to increase the tolerance of a mammal to hypoxia so that ribose and cysteine are elevated in the tissue of the mammal during a hypoxic event.

In accordance with a further aspect of the present invention, there is provided use of RibCys in an effective amount to increase the tolerance of a mammal to hypoxia so that ribose and cysteine are elevated in the tissue of the mammal during a hypoxic event

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the metabolic synthesis of glutathione (GSH) from L-glutanic acid.

Figure 2 depicts the *in vivo* dissociation of a compound of formula I to yield cysteine and an aldehyde.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term RibCys refers to 2(R,S)-D-ribo-(1′,2′,3′,4′-tetrahydroxybutyl) thiazolidine-4(R)-carboxylic acid, as well as the 2R or 2S enantiomers of (Ia), and its pharmaceutically acceptable salts. Such salts include alkali metal salts of the carboxylic acid moiety as well as stable acid addition salts of the NH moiety, including salts of both inorganic and organic acids, such as citrate, malate, gluconate, glutamate, hydrochloride, hydrosulfate and the like.

As used herein, the term "hypoxia" or "hypoxic condition" is defined to mean a condition in which oxygen in one or more tissues of a mammal is lowered below physiologic levels, e.g., to a less than optimal level. Hypoxia also includes conditions in which oxygen levels are lowered in tissues due to stress such as aerobic exercise, physical weight pressure, anesthesia, surgery, anemia, acute respiratory distress syndrome, chronic illness, chronic fatigue syndrome, trauma, burns, skin ulcers, cachexia due to cancer and other catabolic states and the like. Hypoxia also includes "ischemia" or "ischemic conditions" in which tissues are oxygen-deprived due to reduction in blood flow, as due to constriction in, or blockage of, a blood vessel. Ischemia and/or ischemic conditions include those caused by coronary artery disease, cardiomyopathy, including alcoholic cardiomyopathy, angioplasty, stenting, heart surgery such as bypass surgery or heart repair surgery ("open-heart surgery"), organ transplantation, prolonged weight pressure on tissues (pressure ulcers or bedsores), ischemia-reperfusion injury which can cause damage to transplanted organs or tissue, and the like. The present invention is effective to treat the GSH

and ATP depletion due to hypoxia and thus to increase a subject's energy level strength and well-being, even though the underlying cause of the hypoxic condition, such as viral or bacterial infection, exposure to bacterial or other toxins, low red-cell counts, aging, cancer or continued exercise, is not affected.

The term "treating" or "treatment" as used herein includes the effects of RibCys administration to both healthy and patients afflicted with chronic or acute illness and includes inducing protective affects as well as decreasing at least one symptom of a past or ongoing hypoxic condition.

Effective doses of RibCys will vary dependent upon the condition, age and weight of the patient to be treated, the condition to be treated and the mode of administration. Both cysteine, as released *in vivo* from RibCys in animal models, and ribose, as administered directly to human subjects, have been found to be essentially non-toxic over wide dosage ranges. For example, ribose has been reported to increase exercise capacity in healthy human subjects when taken orally at dosages of 8-10 g per day by an adult. See U.S. Pat. No. 6,534,480. RibCys administered to mice at 8 mmol/kg i.p., increased glutathione levels in numerous organs, including heart (1.5x) and muscle tissue (2.5x). See, J.C. Roberts, Toxicol. Lett., 59, 245 (1991). Likewise, RibCys at 8 mmol/kg has been found to deliver effective protective amounts of cysteine to mice exposed to cyclophosphamide. This dose can deliver about 70-80 g of ribose and about 60-70 g of cysteine to an adult human. See J.C. Roberts, Anticancer Res., 14, 383 (1994). Doses of 2 g/kg RibCys were reported to protect mice against acetaminophen hepatic and renal toxicity by A.M. Lucas et al., Toxicol. Pathol., 20, 697 (2000). Doses of 1 g/kg RibCys were reported to protect mice against irradiation-induced bowel injury (see J.K. Rowe et al., Dis. Colon Rectum, 36, 681 (1993). J.E. Fuher (U.S. Patent No. 4,719,201) reported that doses of ribose of about 3 g/day for at least 5 days effectively restored and maintained ATP levels in dogs subjected to ischemia (heart attack model), doses that delivered about 550-700 mg/kg of ribose to an 30 kg dog.

In clinical practice, these compounds, and the pharmaceutically acceptable salts thereof, can be administered in the form of a pharmaceutical unit dosage form comprising the active ingredient in combination with a pharmaceutically acceptable carrier, which can be a solid, semi-solid, or liquid diluent. A unit dosage of the compound can also be administered without a

carrier material. Examples of pharmaceutical preparations include, but are not limited to, tablets, powders, capsules, aqueous solutions, suspensions including concentrates, liposomes, and other slow-releasing formulations, as well as transdermal delivery forms. Typically, the unit dosage form includes about 0.001-99% of the active substance.

The compounds can be delivered by any suitable means, e.g., topically, orally, parenterally. Preferably, the delivery form is liquid or a solid such as a powder that can be stirred into an ingestible liquid. Standard pharmaceutical carriers for topical, oral, or parenteral compositions may be used, many of which are described in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa.

For example, for oral administration, suitable pharmaceutical carriers or diluents can include mannitol, lactose, starch, magnesium stearate, talcum, glucose, and magnesium carbonate. Oral compositions can be in the form of tablets, capsules, powders, solutions, suspensions, sustained release formulations, and the like. A typical tablet or capsule can contain 40-99% lactose, 1-2% magnesium stearate, and 10-20% cornstarch, along with the active substance (preferably about 0.001-20%). An aqueous solution can contain up to the saturation level of RibCys or its salt, preferably with an amount of ribose added that is effective to prevent or inhibit premature *in vitro* dissociation.

For parenteral administration, suitable pharmaceutical carriers can include water, saline, dextrose, Hank's solution, Ringer's solution, glycerol, and the like. Parenteral compositions can be in the form of suspensions, solutions, emulsions, and the like. Parenteral administration is usually by injection or infusion which can be subcutaneous, intramuscular, or intravenous.

Example 1

2(R,S)-D-ribo-1',2',3',4'-Tetrahydroxybutylthiazolidine-4(R)-carboxylic Acid (RibCys).

This compound was synthesized using ribose (Rib) as described by R. Bognar et al., Z. Liebigs Ann. Chem., 738, 68 (1970), the disclosure of which is incorporated by reference herein. The product was collected to give 4.71 g (92.2% yield) of pale yellow material, mp 149°-151° C. dec. $[\alpha]_D^{25}$ -103.1° (c=0.52, H_2O); IR (KBr) *v*3220 (br, OH, COO^-), 1610 cm^{-1} (COO^-).

Example 2

Stimulation of Glutathione Biosynthesis in Isolated Rat Hepatocytes by L-Cysteine Prodrugs and Inhibition by Buthionine Sulfoximine (BSO)

Rat hepatocytes were isolated following the methods of P. O. Seglen, Exper. Cell Res., 74, 450 (1972). After final plating, the hepatocytes were maintained in culture for 24 hr prior to use. Only primary cultures were used throughout the studies. The hepatocytes were incubated with cysteine prodrugs NAC and (Ia) for a 4-hr period, and after removal of media by aspiration, the cells were rinsed with cold phosphate-buffered saline and deproteinized with 5% sulfosalicylic acid. Total GSH content (GSH+GSSG) was determined by a modification of the DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] glutathione reductase recycling method of F. Tietze, Anal. Biochem., 27, 502 (1969). The GSH concentration in the sample was quantified by determining the cycling rate (ΔOD at 412 nm/min) of the sample. For the inhibition studies with BSO, the cells were pre-exposed to BSO (0.20 mM) before treatment with the L-cysteine prodrugs.

The results are shown in Table 1, below:

TABLE 1

Increased Glutathione [GSH] Content of Rat Hepatocytes after Incubation with L-Cysteine Prodrugs

Cysteine Prodrug	Conc. (mM)	[GSH] ± SE ($nmol/10^6$ cells)	[GSH] Rel. to Controls
Control (none)	-	35.4 ± 0.75	1
RibCys (Ia)	1.0	61.2 ± 1.52	1.7
N-Acetyl-L-cysteine (NAC)	2.5	45.8 ± 1.27	1.3

As can be seen from Table 1, RibCys elevated GSH levels about 1.7-fold relative to controls in these hepatocytes. N-Acetyl-L-cysteine (NAC), the drug presently used for the clinical treatment of acetaminophen overdoses, also raised GSH levels by 30% in this system, but required 2.5 times the concentration of the thiazolidine prodrugs for comparable elevation. (See L. F. Prescott et al.,

Brit. Med. J., 2, 1097 (1979); B. J. Lautenburg et al., J. Clin. Invest., 71, 980 (1983) and G. B. Corcoran et al., J. Pharmacol. Exp. Ther., 232, 864 (1985)).

That GSH biosynthesis was stimulated by liberation of its biochemical precursor, L-cysteine, from the prodrugs, was indicated by experiments conducted in the presence of 0.20 mM buthionine sulfoxime (BSO). O. W. Griffith et al., J. Biol. Chem., 254, 7558 (1979), have demonstrated that BSO is a specific inhibitor of gamma-glutamyl cysteine synthetase, the enzyme responsible for catalyzing the first step in GSH biosynthesis. The data summarized on Table 2, below, demonstrate that GSH levels were decreased by this inhibitor even in the presence of RibCys, thus providing evidence that the increased levels of GSH observed were indeed due to de novo GSH biosynthesis from the L-cysteine provided by the thiazolidine prodrugs.

TABLE 2

Inhibitory Effect of Buthionine Sulfoxime (BSO) on GSH Elevation Elicited by L-Cysteine Prodrugs in Rat Hepatocytes

Prodrug (1.0 mM)	BSO (0.2 mM)	[GSH] ± SE (nmol/10^6 cells)	[GSH] Rel. to Controls.
None (Control)	-	35.4 ± 0.78	1.0
None	+	18.4 ± 2.08	0.5
RibCys (1a)	+	16.2 ± 3.60	0.5
N-Acetyl-L-cysteine	+	25.5 ± 1.59	0.7

Example 3

RibCys Elevates GSH in Heart and Muscle Tissue

As reported by J.C. Roberts et al., Toxicol. Lett., 59, 245 (1991), RibCys successfully elevated glutathione (GSH) levels in numerous organs of tumor-bearing CDF1 mice. GSH content was assayed 1, 2, 4, 8 and 16 h after RibCys administration (8 mmol/kg, i.p.); various organs achieved maximal GSH content at different time points. GSH in the liver was elevated 1.5-fold compared to untreated controls at the 16-h time point. Kidney GSH also was maximal at 16 h and achieved 1.6-times control values. GSH in muscle achieved 2.5 times the

levels in control animals, while the bladder was elevated 2.1-fold, and the heart 1.8-fold. Other tissues tested (spleen, pancreas, lung) showed a 1.1- to 1.2-fold increase in GSH content. GSH in implanted L1210 tumors was also elevated only 1.2-fold.

Example 4

Recovery of the Working Canine Heart Following Global Myocardial Ischemia

As reported in Examples 1-2 of J.E. Foker (U.S. Pat. No. 4,605,644), dilute solutions of ribose in normal (0.9%) saline were found effective to decrease the ATP recovery time following myocardial ischemia in the canine model. For example, infusion of a normal saline solution which is 80 mM in ribose at a rate of about 1 ml/min for about 24.0 hours afforded an eight-fold decrease in the ATP recovery time. During this treatment period, about 17.0 g of ribose were introduced into the circulatory system; a total dose of about 550-700 mg ribose/kg of body weight. The appropriate dose for the optimal recovery of ATP levels and cardiac function in a given human subject can be readily established via empirical studies including known assays for ATP levels, cardiac function and the like.

Although the studies of the examples of U.S. Pat. No. 4,605,644 were directed at enhancing the energetic recovery following ischemia of the heart with solutions containing free ribose, the present method employing the cysteine/ribose pro-drug RibCys is also expected to be applicable to any tissue or organ that has suffered hypoxia, such as an ischemic insult where antioxidant augmentation and ATP recovery would be helpful. These situations include but are not limited to: myocardial infarction, stroke, organ transplant with organ preservation, neonatal support, multi-organ system failures, shock and trauma resulting in compromised circulation, and the like. Often, even uncomplicated general anesthesia can result in some degree of hypoxia and the accompanying invasive medical procedure can lead to the build-up of free radicals in the traumatized tissue. Likewise, aerobic exercise in convalescent or healthy individuals can lead to ATP depletion and the build-up of free radicals from environmental oxidants. Therefore, the present invention provides a method whereby hypoxic tissue can be treated so as to quickly regain and maintain

normal ATP levels, both to improve tissue survival and to hasten general bodily recovery.

While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth in the examples, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the scope of the invention.

The embodiments of the present invention in which an exclusive property or privilege is claimed are defined as follows:

1. Use of RibCys or a pharmaceutically acceptable salt thereof in the preparation of a medicament to effectively maintain, restore or increase both the ATP levels and the glutathione levels so as to treat hypoxia in a mammal threatened with, or afflicted with, a hypoxic condition.

2. The use according to claim 1, wherein the hypoxia is due to an ischemic insult.

3. The use according to claim 2, wherein the ischemic insult is produced during heart surgery, organ transplantation, angioplasty or stenting.

4. The use according to claim 2, wherein the ischemic insult is due to cardiovascular disease, cardiomyopathy, myocardial stunning, peripheral vascular disease, intermittent claudication, tachycardia or ischemia-reperfusion.

5. The use according to claim 1, wherein the hypoxia is due to an anesthesia, physical body weight pressure, septicemia, stroke, a surgical procedure, burn, pulmonary dysfunction, physical exertion or chronic illness.

6. Use of RibCys or a pharmaceutically acceptable salt thereof to effectively maintain, restore or increase both the ATP levels and the glutathione levels so as to treat hypoxia in a mammal threatened with, or afflicted with, a hypoxic condition.

7. The use according to claim 6, wherein the hypoxia is due to an ischemic insult.

8. The use according to claim 7, wherein the ischemic insult is produced during heart surgery, organ transplantation, angioplasty or stenting.

9. The use according to claim 7, wherein the ischemic insult is due to cardiovascular disease, cardiomyopathy, myocardial stunning, peripheral vascular disease, intermittent claudication, tachycardia or ischemia-reperfusion.

10. The use according to claim 6, wherein the hypoxia is due to an anesthesia, physical body weight pressure, septicemia, stroke, a surgical procedure, burn, pulmonary dysfunction, physical exertion or chronic illness.

11. Use of an amount of 2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof in the preparation of a medicament to effectively maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in a tissue in a mammal subject to hypoxia.

12. The use according to claim 11, wherein the mammal is a human.

13. The use according to claim 1, 6, 11 or 12, wherein the RibCys is for oral administration.

14. The use according to claim 1, 6, 11 or 12, wherein the mammal was, is or will be subjected to ischemia insult.

15. The use according to claim 14, wherein the ischemia insult is to cardiovascular tissue.

16. The use according to claim 15, wherein the cardiovascular tissue is myocardial tissue.

17. The use according to claim 14, wherein said ischemic insult is produced during heart surgery, organ transplantation, angioplasty or stenting.

18. The use according to claim 16, wherein the ischemia is due to cardiovascular disease, cardiomyopathy, myocardial stunning, peripheral vascular disease, intermittent claudication, tachycardia or ischemia-reperfusion.

19. The use according to claims 1, 6, 11 or 12, wherein the hypoxia is due to an anesthesia, physical body weight pressure, septicemia, stroke, a surgical procedure, burn, pulmonary dysfunction, physical exertion or chronic illness.

20. The use according to claim 19, wherein the body weight pressure causes pressure ulcers.

21. Use according to claim 19, wherein the chronic illness is due to viral infection.

22. Use according to claim 21, wherein the viral infection is due to HCMV, HIV or EBV.

23. Use according to claim 19, wherein the chronic illness is due to bacterial infection.

24. Use according to claim 19, wherein the chronic illness is cancer.

25. Use of an amount of 2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof to effectively maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in a tissue in a mammal subject to hypoxia.

26. The use according to claim 25, wherein the mammal is a human.

27. The use according to claim 25 or 26, wherein the RibCys is for oral administration.

28. The use according to claim 25 or 26, wherein the mammal was, is or will be subjected to ischemia insult.

29. The use according to claim 28, wherein the ischemia insult is to cardiovascular tissue.

30. The use according to claim 29, wherein the cardiovascular tissue is myocardial tissue.

31. The use according to claim 28, wherein said ischemic insult is produced during heart surgery, organ transplantation, angioplasty or stenting.

32. The use according to claim 30, wherein the ischemia is due to cardiovascular disease, cardiomyopathy, myocardial stunning, peripheral vascular disease, intermittent claudication, tachycardia or ischemia-reperfusion.

33. The use according to claim 25 or 26, wherein the hypoxia is due to an anesthesia, physical body weight pressure, septicemia, stroke, a surgical procedure, burn, pulmonary dysfunction, physical exertion or chronic illness.

34. The use according to claim 33, wherein the body weight pressure causes pressure ulcers.

35. Use according to claim 33, wherein the chronic illness is due to viral infection.

36. Use according to claim 35, wherein the viral infection is due to HCMV, HIV or EBV.

37. Use according to claim 33, wherein the chronic illness is due to bacterial infection.

38. Use according to claim 33, wherein the chronic illness is cancer.

39. Use of RibCys in the preparation of a medicament in an effective amount to increase the tolerance of a mammal to hypoxia so that ribose and cysteine are elevated in the tissue of the mammal during a hypoxic event.

40. The use according to claim 39, wherein the mammal is a human.

41. The use according to claim 1, 6, 11, 12, 25, 26, 39 or 40, wherein RibCys is for administration in a dosage of about 10 to about 150 grams.

42. The use according to claim 1, 6, 11, 12, 25, 26, 39 or 40, wherein the RibCys or salt thereof is for administration at least five minutes prior to the occurrence of the hypoxic event.

43. The use according to claim 1, 6, 11, 12, 25, 26, 39 or 40, wherein the RibCys or salt thereof is for administration in a liquid vehicle comprising an amount of free ribose effective to inhibit *in vitro* dissociation of RibCys prior to administration.

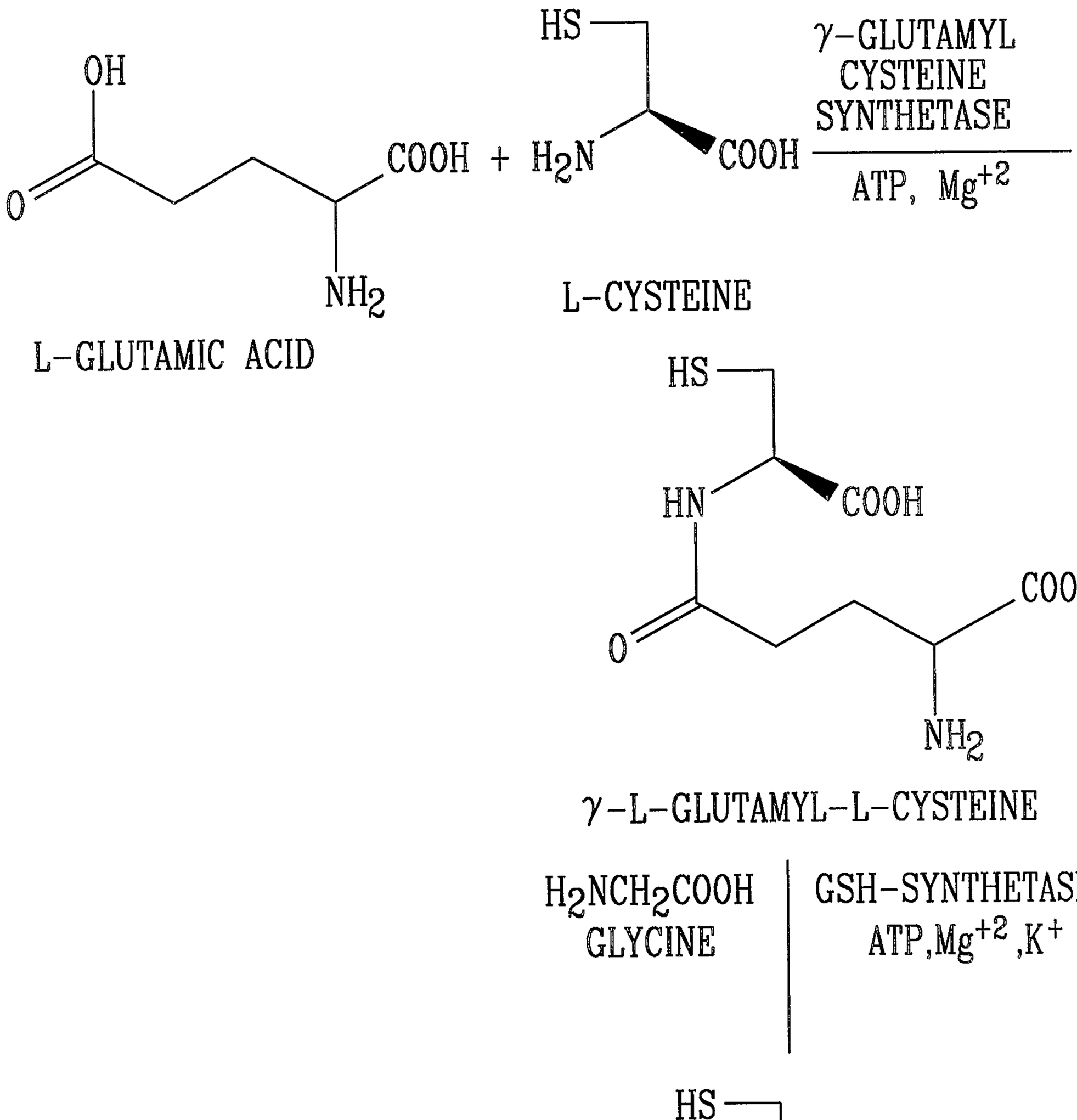
44. Use of RibCys in an effective amount to increase the tolerance of a mammal to hypoxia so that ribose and cysteine are elevated in the tissue of the mammal during a hypoxic event.

45. The use according to claim 44, wherein the mammal is a human.

46. The use according to claim 44 or 45 wherein RibCys is for administration in a dosage of about 10 to about 150 grams.

47. The use according to claim 44 or 45, wherein the RibCys or salt thereof is for administration at least five minutes prior to the occurrence of the hypoxic event.

48. The use according to claim 44 or 45, wherein the RibCys or salt thereof is for administration in a liquid vehicle comprising an amount of free ribose effective to inhibit *in vitro* dissociation of RibCys prior to administration.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HS
HN
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COOH
O
NH2
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FIG. 1

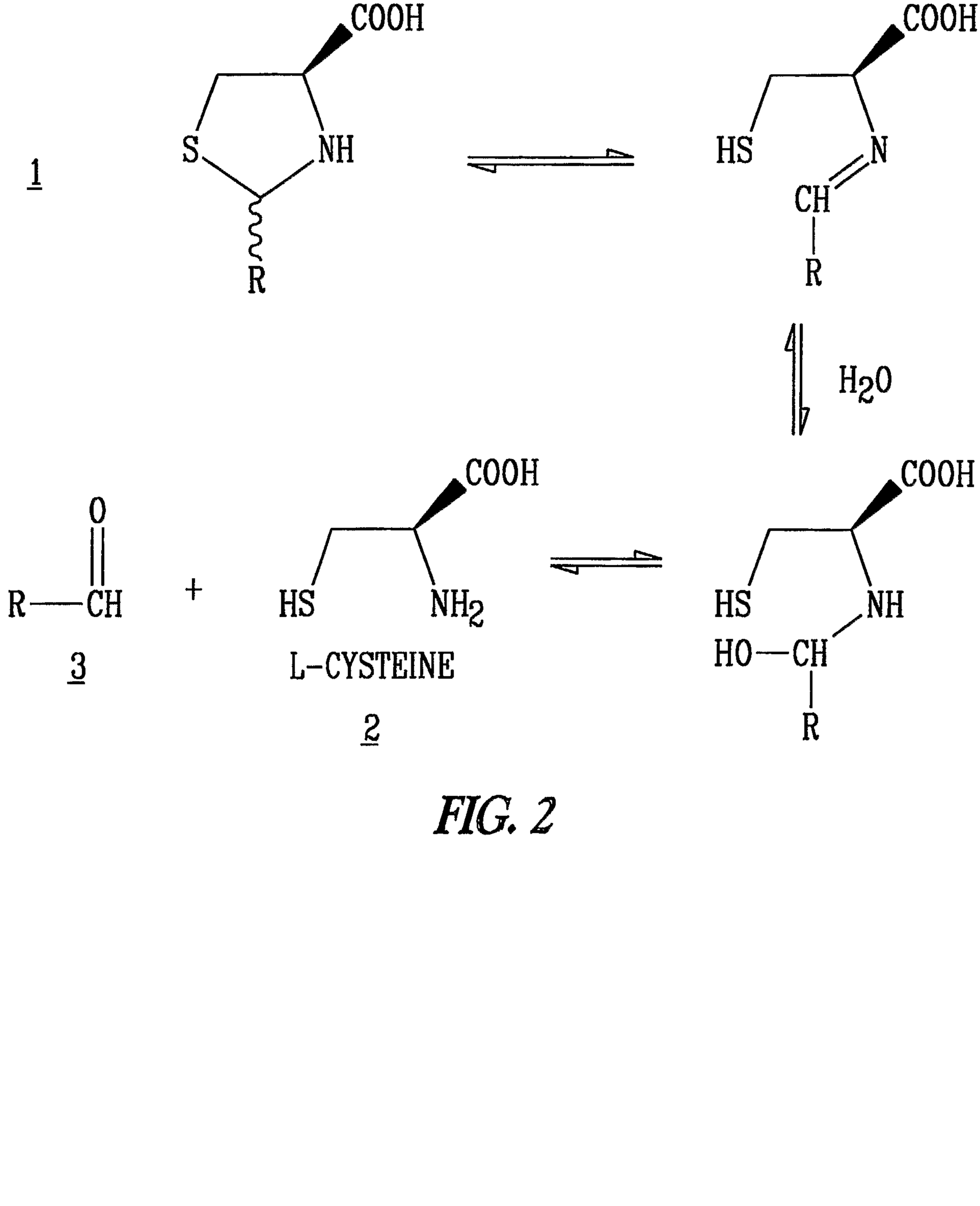
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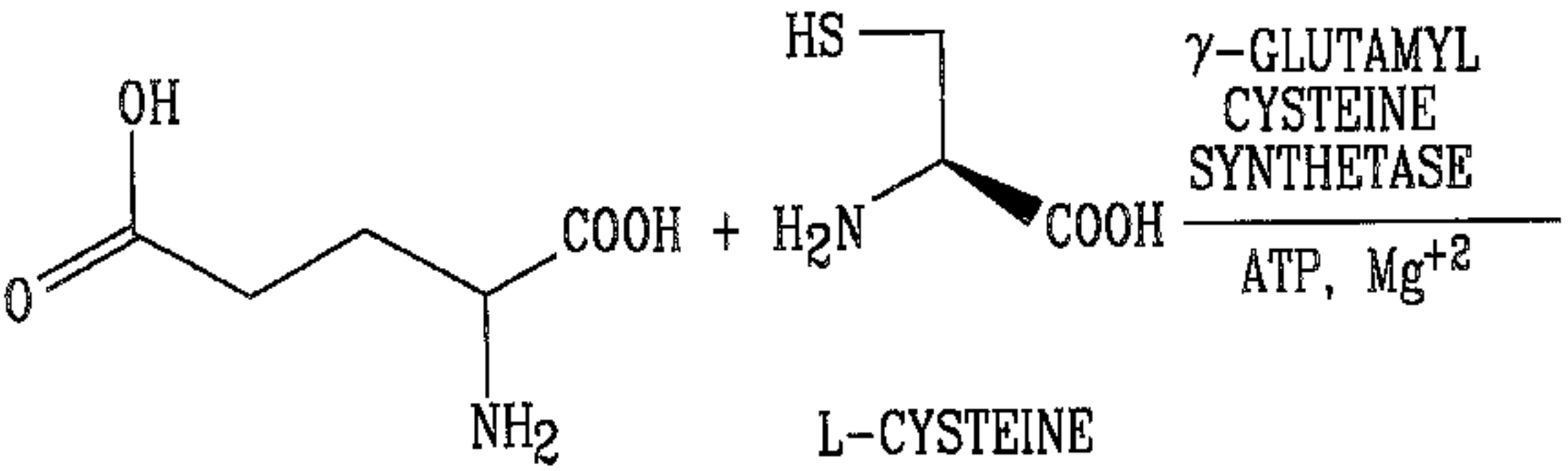
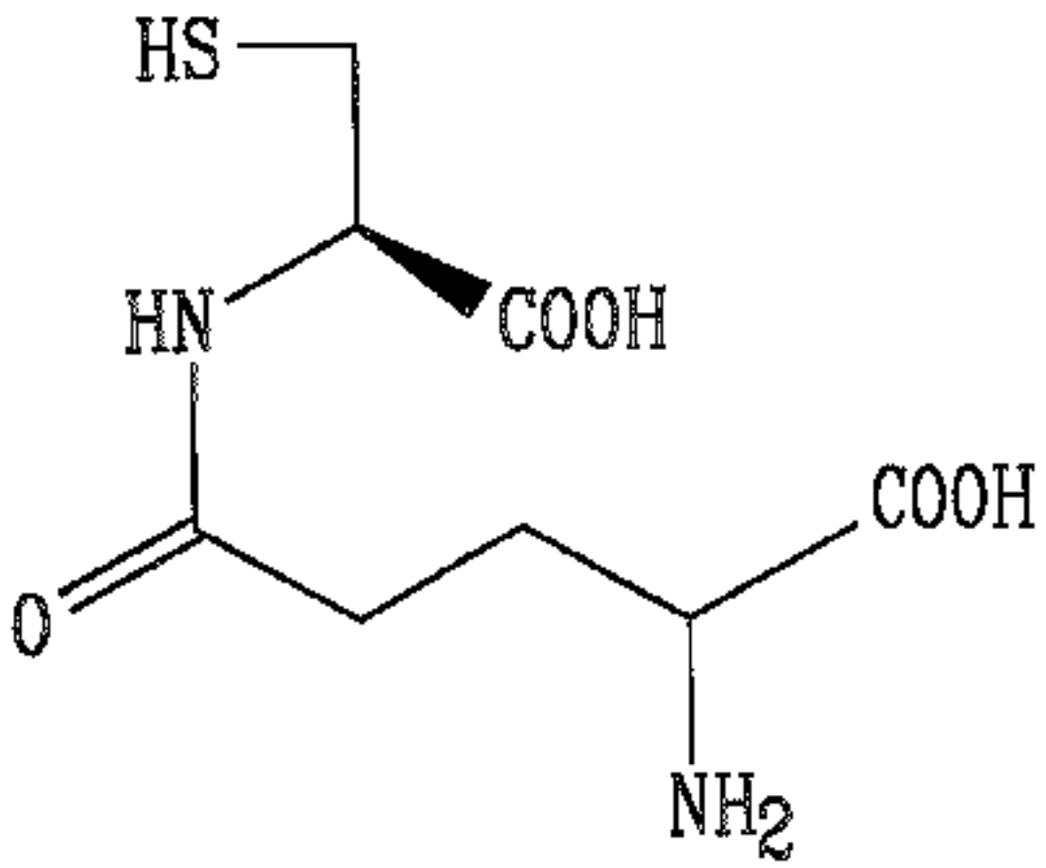
FIG. 2





γ-L-GLUTAMYL-L-CYSTEINE

H_2NCH_2COOH
GLYCINE

GSH-SYNTHETASE
ATP, Mg^{+2}, K^+