Artículos de Revisión

Interrelationship between GSH and ascorbate in mammalian cells: Physiological and Clinical implications

Interrelaciones entre GSH y ascorbato en células de mamífero: Implicaciones fisiológicas y clínicas

GARCÍA, C., BARBER, T., PUERTES, I., MURGUI, A. and VIÑA, J. R.
Departamento Bioquímica y Biología Molecular. Facultades de Medicina y Farmacia. Av. Blasco Ibáñez, 17. Universitat de Valencia. Valencia-46010. SPAIN.

RESUMEN

Las vitaminas poseen diferentes papeles fisiológicos y clínicos además del de la prevención de enfermedades carenciales. Este es el caso de la vitamina C, que más allá de prevenir el escorbuto, posee actividad antioxidante bien descrita y la capacidad de ahorrar GSH. El GSH es un tripéptido ampliamente distribuido en las células de mamíferos, el cual no es requerido en la dieta. El ciclo del gamma glutamilo es el responsable de la síntesis y de la degradación del GSH. Este tripéptido provee a la célula de un medio reductor a través de la acción de la glutation difulfuro reductasa. La administración de ácido ascórbico puede contribuir también al sistema reductor de las células. Existen numerosos datos científicos que apoyan el hecho de que algunas condiciones asociadas con estrés oxidativo podrían ser mejoradas por una terapia que mantuviera los niveles normales de GSH. Esto se puede conseguir por la administración de ésteres de GSH, aumentando la capacidad de síntesis de GSH al proporcionar los substratos, como el N-acety-L-cysteina y/o aumentando la disponibilidad de compuestos como el ascorbato que puede ahorrar GSH. Todos estos efectos podrían ser de interés clínico para el diseño de un "cocktail" adecuado que mantuviera el GSH intracelular dentro de valores normales para tejidos de mamíferos, en condiciones en las que el GSH estuviera disminuido.
Palabras clave: Glutation. Ascorbato. Células de mamífero.

ABSTRACT

Vitamins have different physiological and clinical roles besides preventing deficiency diseases. This is the case of vitamin C that beyond preventing scurvy, it has a well known antioxidant activity and the capacity to spare GSH. GSH is a tripeptide widely distributed in mammalian cells, which is not required in the diet. The gamma-glutamyl cycle is responsible for the synthesis and degradation of GSH. This tripeptide provides the cell with a reducing milieu that is achieved through the action of glutathione disulfide reductase. Administration of ascorbic acid may also contribute to the reducing properties of cells. There is enough scientific background to support the fact that several conditions associated with oxidative stress might be improved by therapy that maintain GSH within

normal leves. This can be achieved by the administration of GSH-esters, increasing the capacity for GSH synthesis by providing substrates such as N-acetyl-L-cysteine and/or by increasing the availibility of compounds such as ascorbate that can spare GSH. All these facts could be of clinical interest in the design of the right "cocktail" in order to keep intracellular GSH within normal values in mammalian tissues under those situations were GSH is depleted.
Key words: Glutathione. Ascorbate. Mammalian cells.

Recibido: 15-6-1994.
Aceptado: 25-7-1994.
BIBLID [0004-2927(1994) 35:2; 261-275]

INTRODUCTION

Since the beginning of this century, the interest in vitamins has been based on their role in preventing vitamin-deficiency diseases. However, in the last decade, an enormous amount of information has been gathered which shows that vitamins have different physiological and clinical roles beyond preventing deficiency diseases [Machlin, 1992; Gershoff, 1993].

The diet must provide daily the minimum of ascorbic acid (vitamin C) necessary to prevent scurvy, a disease characterized by a reduced stabililty of connective tissue. Skin, bone and tendon are the tissues more affected by ascorbate deficiency. Beyond its role in preventing scurvy, ascorbic acid has different physiological roles. Recent studies in the ability of ascorbic acid to spare GSH will be the basis for much of this review.

Ascorbic acid is considered the most important antioxidant in extracellular fluids because exposure of plasma to aqueous peroxyl radicals generated at a constant rate, leads to oxidation of endogenous ascorbate and sulfhydryl groups, followed by sequential depletion of bilirrubin, urate and alfa-tocopherol [Frei *et al.*, 1988]. Other physiological function of ascorbate is the ability to spare GSH [Mårtensson & Meister, 1991] . This could be of potential interest in the design of the right "cocktail" in order to keep intracellular GSH within normal values in mammalian tissues under those situations were GSH is depleted, such as paracetamol intoxication [Mitchell, 1974] or obesity [Sastre *et al.*, 1989].

FUNCTIONS OF VITAMIN C

Nearly all animals can synthesize ascorbic acid from D-glucose except for some species such as humans, nonhuman primates, guinea pigs, indian fruit bats and bulbuls [Levine 1986]. These animals can perform the reactions needed for the biosynthesis of ascorbic acid from D-glucose except the last step that is

catalysed by the L-gulono-gamma-lactone oxidase. In these species, this enzyme is missing because of a mutation; therefore the need for vitamin C in the diet is the result of an inborn error in carbohydrate metabolism [Wilson, 1994]. These animals survive because membrane transport allows dietary ascorbate to be absorbed as chyme moves through the small intestine [Rose & Bode, 1993].

Adult rats can synthesize ascorbic acid; however, there is indirect evidence that newborn rats must be included in the group of animals that cannot synthesize ascorbic acid, because newborn rats are more sensitive than adult rats to GSH deficiency and their dependence on GSH seems to be similar to that of guinea pigs [Mårtensson & Meister 1991].

Ascorbic acid has several physiological functions beyond its role in collagen synthesis [Devesa *et al.,* 1993 a; Gershoff, 1993]. The vitamin reduces the prosthetic metal ions in many enzymes to the correct form and performs other antioxidant functions by removing free radicals. The concept that ascorbate is a reductant of other molecules in the body was introduced by Szent-Gyorgi in 1928. Recently, it has been proposed that there are three reasons for suggesting that ascorbate serves as scavenger of free radicals in human body: i) it is chemically suited to react with oxidizing free radicals, ii) it is present at sufficient high concentration in the body to be effective, iii) it fits into the physiology of cellular transport and metabolism [Rose & Bode, 1993].

Paradoxically, in the presence of Fe^{3+} or Cu^{2+}, ascorbate can promote the generation of the same reactive oxygen species ($OH^{\cdot}$, O^{2-}, H_2O_2, and ferryl ion) it is known to destroy. The limiting factor for the pro-oxidant activity is most likely to be the availability of metal ions rather than the physiological levels of ascorbate. Although the total iron concentration of most tissues is quite high, iron exists almost entirely tightly sequestered in protein complexes, thus, it is not readily available for oxygen radical generation. Megadoses of ascorbic acid may promote the oxidation of serum lipids in iron loaded animals [Miller & Kapsokefalou, 1994].

Vitamin C is also required for the following cellular roles: i) iron and copper nutrition, ii) synthesis of carnitine, iii) synthesis of noradrenaline, iv) peptide alpha-amidation, v) degradation of cholesterol and L–tyrosine, vi) the recycling of 5,6,7,8, tetrahydrobiopterin, vii) normal chondrocyte physiology, viii) GSH sparing effect, ix) protection against low density lipoprotein oxidation and x) antiviral activity.

Vitamin C has also been claimed to have anticataract action and a "randomized trial of vitamin supplementation in cataract prevention may well be justified" [Robertson *et al.,* 1991]. The protective effects on cancer have been also claimed but the evidence in humans is based on epidemiologic studies [Byers & Perry, 1992]. It also important to emphasize that animal and cell-culture studies have been performed trying to show a protective role for ascorbic acid in cancer; but the fact that some of these experiments are faulted should

not encourage us to think that the hypothesis should not be valid [Gershoff, 1993].

FUNCTIONS OF GLUTATHIONE (GSH)

GSH (L-glutamyl-L-cysteinyl-glycine) is a tripeptide widely distributed in mammalian cells and essential for cell physiology. Many functions of GSH are derived from its special chemical properties: it contains a thiol group and a ∂-glutamyl linkage that is resistant to degradation by the usual peptidases.

The gamma-glutamyl cycle is responsible for the synthesis and degradation of GSH [Meister, 1991; Figure 1]. The synthesis is achieved by the consecutive actions of the ∂-glutamyl-cysteine synthetase and GSH synthetase. The former enzyme functions at less than maximal rate due to feedback inhibition by GSH. The tripeptide is exported out of the cell; therefore, the breakdown of GSH occurs extracellularly and is catalysed by the ∂-glutamyltranspeptidase (GGT) and dipeptidases bound to the external surfaces of cell membranes. Transpeptidation occurs in the presence of some amino acids such as L-methionine, L-alanine, L-cysteine, L-glutamine, and L-serine, and leads to the formation of ∂-glutamyl amino acids. These compounds are transported into the cells and they are substrates of the intracellular enzyme ∂-glutamylcyclotransferase, which converts ∂-glutamyl amino acids into 5-oxoproline and the corresponding free amino acids. 5-oxoproline is converted to glutamate in a reaction catalyzed by 5-oxoprolinase (Figure 1).

GSH has a protective role, several metabolic functions and a role in transport processes. The protective role is due to several actions such its role in drug detoxification, in their protection against oxidative stress and radiation [Meister, 1991] and in the normal functioning of $CD4^{+-}$ T cell [Kinscherf, 1994]. In experimental animals, GSH protects against caerulin-induced pancreatitis and against the myoglobinuric renal failure.

The metabolic functions are the following: i) role in DNA synthesis, ii) as a reservoir of L-cysteine, iii) as a methionine-sparing compound, iv) in the regulation of pentose–phosphate pathway and gluconeogenesis, v) in the maintenance of cell membrane thiol status, vi) in the deiodination of thyroid hormone, vi) as a coenzyme, vii) in the formation of disulfide bonds in a protein, viii) in the leukotriene synthesis, viii) in calcium homeostasis, ix) as a methionine-sparing compound, x) role in the ageing of cells and xi)sparing of ascorbate by GSH xii) role in secretion of polypeptide hormones and in neurotransmitter release (For details see Meister, 1994, Viña *et al.,* 1989 b).

The gamma-glutamyl cycle has been involved in amino acid uptake by cells with high GGT activity (For review see Meister, 1989); however, we have evidence that this cycle should not be considered a mechanism for amino acid

transport but rather a generator of extracellular signals, ∂-glutamyl-amino acids, that are converted intracellularly to 5-oxoproline, which activates the uptake and/or metabolism of amino acids (Viña *et al.,* 1989 a).

There is a flow of GSH (rather than of glutathione disulfide) from tissues with low transpeptidase (GGT) to plasma, and from plasma to those tissues with high GGT activity. Plasma levels of GSH are markedly increased after administration of GGT inhibitors and treatment of rats with L-buthionine-(S,R)-sulfoximine, a selective inhibitor of the ∂-glutamyl-cysteine synthetase, leads to a rapid and marked decrease in the levels of plasma GSH; therefore, there is an active turnover of plasma GSH.

The major organs involved in this intertissue flux of GSH are the liver, which has very low activity of GGT, and the kidney, pancreas, lung, lactating mammary gland, that are tissues with high activity of GGT [Meister, 1984]. In the kidney of adult rats, GGT is located mainly on the tubular side (Curto *et al.,* 1988) but there is also GGT activity on the basolateral side of renal cells. The kidney removes about two-thirds of the plasma GSH and the remainder by extrarenal transpeptidase (Figure 2). Most of the renal tubular glutathione arises from kidney cells in an intraorgan GSH transport process.

This intertissue flux of GSH has several physiological functions and among them it is important to emphasize the fact that it is an efficient way of L-cysteine moieties delivery from the liver to the peripheral tissues.

INTERRELATIONSHIP BETWEEN GSH AND ASCORBATE.

Standard reduction potentials of free radicals and antioxidants

Free radicals --chemicals species containing one o more unpaired electrons– are produced in the body constantly by normal metabolic processes. Oxidative "attack" by free radicals may lead to damage to proteins, DNA, membranes and lipoproteins. As a consecuence of the deleterious effects of these oxygen–derived species, free radicals are thought to be involved in a number of pathological processes including atherogenesis, neoplasia and aging. Organisms have developed many defenses to protect themselves from free radical processes. Antioxidants may be classified as either enzymatic or non–enzymatic and occur at different locations within the cell, in the membrane or in the extracellular environment. Free radicals vary widely in their thermodynamic properties, ranging from very oxidizing to very reducing. The biochemical standard one–electron reduction potentials ($\varepsilon^{\circ\prime}$) (Table 1) are of interest for predicting the direction of free radicals processes and correlate with experimental data (Buettner, 1993). Since electrons spontaneously flow from low to high reduction potentials, they are transferred, under standard conditions, from the reduced products in any half

reaction to the oxidized reactants of any half–reaction above it (although this may not occur at a measurable rate in the absence of a suitable enzyme). Thus, the ε°' are listed in order from highly oxidizing to highly reducing species.

However, it must kept in mind that reactions other than electron / hydrogen atom transfer are possible and that $\Delta\varepsilon^{\circ\prime} < 0$ does not make a reaction impossible. The hierarchy of free radical reactions presented in Table 1 allows us to predict the cascade of free radical reactions that will occur after initiation (Figure 3). Thus, ascorbate and tocopherol are both well–suited to serve a small molecule chain breaking donor antioxidants in biological systems. Once membrane lipid peroxidation is initiated, the peroxyll radical is converted to a lipid hydroperoxide (thereby preventing chain propagation reaction) and the resulting tocopheroxyl radical can be repaired by ascorbate ($AscH^- + TO^{\cdot} \rightarrow Asc^{\cdot -} + TOH$) ($\Delta\varepsilon^{\circ\prime} \approx +200$ mV). The ascorbate radical can be removed by dismutation (yielding $AscH^-$ and dehydroascorbate). Both species can be reduced by enzyme systems and ascorbate is recycled.

Glutathione functions as preventive antioxidant, by reducing hydrogen peroxide and other peroxides (involved in the initiation of free radical chain reactions) in processes catalyzed by glutathione peroxidases. Also acts a chain–breaking antioxidant, "repairing" oxidizing radicals directly : it reacts with various highly oxidizing species as $HO^{\cdot}$, $RO^{\cdot}$, or $ROO^{\cdot}$ generating H_2O, ROH or ROOH + $GS^{\cdot}$ ($\Delta\varepsilon^{\circ\prime} > 0$). The radical $GS^{\cdot}$ is less oxidizing than the other; however, it can react with another GSH via GS^- yielding a strongly reducing species, $GSSG^{\cdot -}$,($GS^{\cdot} + GS^- \rightarrow GSSG^{\cdot -}$). The very negative potential of the GSSG /$GSSG^{\cdot -}$ couple ($\Delta\varepsilon^{\circ\prime} \approx -1500$ mV) (See Table 1) makes $GSSG^{\cdot -}$ the most reducing species that can arise in a biological setting. This species can react with O_2 and produce $O_2^{\cdot -}$ + GSSG, making superoxide dismutase and GSH, in combination, an important and integral component of cellular antioxidant defense.

In addition, glutathione also functions as part of the system for the protection of cells membranes connected with the maintenance of α–tocopherol and ascorbic acid in the reduced state. Thus, administration of ascorbic acid can increase the availability of glutathione for various functions by sparing the glutathione requirement for the reduction of dehydroascorbic acid.

On the enzymology of this process

Ascorbate interacts with free radicals and protects the cells, but undergoes an oxidation to dehydroascorbate. However, to restore the antioxidant potential offer by ascorbate, cellular systems that are capable to reduce back dehydroascorbate to ascorbate must be present. In fact, there is a classic study showing that the administration of dehydroascorbate can prevent scurvy, which indicates that reduction of dehydroascorbate acid to ascorbic acid takes place "in vivo" [Meister,

1992]. This reduction must be efficient since dehydroascorbate is degraded rapidly and irreversibly [Borsook *et al.,* 1937]. Indeed, several proteins such a porcine liver thioltransferase (glutaredoxin, which is localized in the cytosol), a bovine thymus and a human placenta thioltransferase (glutaredoxin) and a highly purified bovine liver microsomal protein disulfide isomerase have glutathione–dependent dehydroascorbate reductase activity [Wells *et al.,* 1990].

However, it has been found recently in liver a protein with dehydroascorbate-reductase activity that requires NADPH-H^+ and not GSH [Casini *et al.,* 1993]. In brain it also has been shown that GSH and NADPH-H^+ are both effective in promoting dehydroascorbate reduction; furthermore, in this study it was also showed that glutathione reductase did not account for the dehydroascorbate reduction [Rose, 1993].

It is also important to consider that in platelets the uptake of dehydroascorbate was greater than that of ascorbic acid and the dehydroascorbate was quickly reduced within platelets [Hornig *et al.,* 1971]. Recently using *Xenopus laevis* oocyte expression it has been shown that mammalian facilitative hexose transporters are a physiologically significant pathway for the uptake and accumulation of ascorbic acid against a concentration gradient (Vera *et al.,* 1993).

Studies in experimental animals

The administration of buthionine-(S,R) sulfoximine (BSO), an inhibitor of the gamma-glutamylcysteine synthetase, produced a glutathione deficiency in newborn rats and this was followed by a significant decrease in ascorbate levels of kidney, liver, brain and lung. These rats had a very high mortality rate; however, administration of large doses of ascorbate decreased mortality, the levels of ascorbic acid were back to normal values and GSH was spared [Mårtensson & Mesiter, 1991].

The induction of GSH deficiency with BSO to newborn rats and guinea pigs is incompatible with life; however, adult mice are able to survive such deficiency because they can synthesize ascorbate [Mårtensson & Meister, 1992]. This support the fact that a consequence of GSH deficiency in mice is the induction of ascorbic acid synthesis in the liver [Meister, 1992, 1994]. This is not case of newborn rats, which support the hypotheses that ascorbate is not synthesized in the newborn rats.

The acute toxicity of BSO in guinea pigs was related with depletion of both cytosolic and mitochondrial pools of GSH in liver and kidney. When several doses of BSO were injected to guinea pigs, it was shown that lethality correlates with depletion of mitochondrial GSH in liver and kidney [Griffith *et al.,* 1991]. In the same study, it was shown that the mortality of the guinea pigs depleted with BSO is associated with mitochondrial swelling and degeneration, causing

proximal tubule necrosis in kidney and focal necrosis in liver. This was accompanied in the lung by swelling of type 2 cell lamelar bodies and of capillary endothelium. Therefore mortality is attributed to multiple organ failure, playing a key role the liver and kidney [Griffith *et al.,* 1991].

The ascorbate concentration in the brain is quite high [Milby and Adams, 1982], only matched in mammalian tissues by the adrenal medulla [Grünewald, 1993]. Ascorbic acid is concentrated in the brain via CSF and not from the blood-brain barrier. The apperance of ascorbic acid into the CSF, which is at equilibrium with the extracellular space of the brain [Spector, 1981], occurs at the choroid plexus, through a carrier-mediated mechanism. The ascorbate is taken up from the extracellular space and it is concentrated in the neuropil. Using primary astrocyte cultures the apparent Km for ascorbate uptake was 32 μM in 138 mM Na^+ [Wilson, 1989]. However, it is surprising that ascorbate could not be detected in cultured astrocytes [Raps *et al.,* 1989]. The fact that neuronal somata do not appear to contain appreciable amounts of GSH [Philbert *et al.,* 1991], which is confirmed by the fact that neurons in culture have very low GSH [Raps *et al.,* 1989], points out the importance of maintaining GSH in astrocytes. These cells showed in culture a narrow range of cellular size but a wide range of extracellular GSH which was resolved into three distinct subpopulations which represent 20%, 35% and 45% of total astrocyte number [Devesa *et al.,* 1993 b].

Incubation of astrocytes with tert-butyl hydroperoxide (t-booH) decreased GSH concentration. This reduction was improved in part by the addition of ascorbate [Viña *et al.,* 1992] or dehydroascorbate. When L-cystine and ascorbate were added together to the t-booH-treated astrocytes, the GSH concentration was indistinguishable from controls [Viña *et al.,* unpublished results].

Studies in humans

GSH is an important antioxidant because helps in the destruction of hydrogen peroxide and lipid peroxides due to the fact that it is substrate of the glutathione peroxidase and promotes the formation of the reduced forms of other antioxidants such us ascorbate [Meister, 1992].

After 60 days of low ascorbic acid intake, the plasma concentration of ascorbic acid in humans was lower than 6 μmol/L and the total glutathione and the reduced glutathione/oxidized glutathione ratio was decreased in plasma [Henning *et al.,* 1991]. Moreover, in healthy adults, supplementation with 500 mg/d of ascorbic acid significantly raised red blood cell GSH concentration and improved the overall antioxidant protection capacity of blood [Johnston *et al.,* 1993].

RECOMMENDED DIETARY ALLOWANCES FOR ASCORBATE

The subcommittee on the Tenth Edition of the RDAs (National Research Council, USA)(1989) has set the RDA for adult men and women at 60 mg/day. This value is proposed for the RDA based upon (a) the depletion and turnover rates, (b) the average depletion rates and steady state turnover rates at a pool size of 1500 mg, (c) an absorption of 85% for usual intakes and (d) the loss of ascorbic acid in food processing. This level of intake will prevent signs of scurvy for at least four weeks. An additional allowances of 10 mg/day and 35 mg/day increment in the maternal vitamin C RDA is recommended during pregnancy and the first six months of lactation, respectively.

Such a wide range between the minimum amount of a nutrient needed to prevent a deficiency disease and the recommended intake is unusual. Several considerations have convinced nutritionists that the RDA for vitamin C should be higher [Gershof, 1993]. The fact that vitamin C has different physiological functions beyond preventing scurvy, such as the capacity to behave as an antioxidant and to spare glutathione should be taken into account in future editions of the RDA. It has been shown that the average ascorbic acid intake estimated to maximize the total body pool was 138 mg/dl [Jacob *et al.,* 1987].

CONCLUDING REMARKS

Vitamins have different physiological and clinical roles besides preventing deficiency diseases. This is the case of vitamin C that beyond preventing scurvy, it has a well known antioxidant activity and the capacity to spare GSH.

GSH is a tripeptide widely distributed in mammalian cells, which is not required in the diet. The gamma–glutamyl cycle is responsible for the synthesis and degradation of GSH. This tripeptide provides the cell with a reducing milieu that is achieved through the action of glutathione disulfide reductase (Figure 1). Administration of ascorbic acid may also contribute to the reducing properties of cells.

There is enough scientific background to support the fact that several conditions associated with oxidative stress might be improved by therapy that maintain GSH within normal leves. This can be achieved by the administration of GSH–esters, increasing the capacity for GSH synthesis by providing substrates such as N–acetyl–L–cysteine and/or by increasing the availibility of compounds such as ascorbate that can spare GSH. All these facts could be of clinical interest in the design of the right "cocktail" in order to keep intracellular GSH within normal values in mammalian tissues under those situations were GSH is depleted.

ACKNOWLEDGMENTS

We want to thanks the grants to Juan R. Viña (DGICYT PM 91–0198; FISS 94–1573) and to Teresa Barber (FISS 92–0263).

REFERENCES

BUETTNER, C. R.: "The pecking order of free radicals and antioxidants: Lipid peroxidation, α– tocopherol and ascorbate". *Arch. Biochem. Biophys.* 1993; 300: 535–543.

BYERS, T. & PERRY, G.: "Dietary carotens, vitamin C, and vitamin E as protective antioxidants in human cancers". *Ann. Rev. Nutr.* 1992; 12: 139-159.

BORSOOK, H., DAVENPORT, H. W., JEFFREYS, C. E. P. & WARNER, R. C.: "The oxidation of ascorbic acid and its reduction *in vitro* and *in vivo*". *J. Biol. Chem.* 1937;117: 237-279.

CASINI, A. F., MAELLARO, E., DEL BELLO, B., SUGHERINI, L,. SANTUCCI, A., COMPORTI, M.: "Redox cycle of ascorbate in the liver: A study of dehydrascorbate-reductase activities". In: *Program & Abstract Book, Society for Free Radical Research (Summer Meeting),* 1993; Abstract n.º 5.5, Siena, Italy.

CURTO, K. A., SWEENEY, W. E., AVNER, E. D., PIESCO, N. P., & CURTHOYS, N. P.: "Immunocytochemical localization of ∂-glutamyltranspeptidase during fetal development of mouse kidney. J. Histochem". *Cytochem.* 1988: 36:159-164.

DEVESA, A., GARCÍA, C., JUAN, G., GUASCH, R. M., O'CONNOR, J. E., & VIÑA, J. R.: "Ascorbic acid: The antioxidant role and its relationship with glutahione". *J. Clin. Nutr. Gastroenterol.* 1993a; 8:35-39.

DEVESA, A., O'CONNOR, J. E., GARCÍA, C., PUERTES, I. R. & VIÑA, J. R.: "Glutathione metabolism in primary astrocyte cultures: flow cytometric evidence of heterogeneous distribution of GSH content". *Brain Res.* 1993 b; 618:181-189.

FREI, B., ENGLAND, K., AMES, B. N.: "Ascorbate in an out–standing antioxidant in human blood plasma". *Proc. Natl. Acad. Sci.* 1989: 86:6.377–6.381.

GERSHOFF, S. N.: "Vitamin C (ascorbic acid): New roles, new requirements". *Nutr. Rev.* 1993; 51:313-326.

GRIFFITH, O. W., HAN, J. & MÅRTENSSON, J.: "Vitamin C protects adult guinea pigs against tissue damage and lethality caused by buthionine sulfoximine-mediated glutahione depletion". *FASEB J.* 1991, 5, Abstract n.º 4708.

GRÜNEWALD, R. L.: "Ascorbic acid in the brain". *Brain. Res. Rev.* 1993; 18:123-133.

HENNING, S. M., ZHANG, J. Z., MCKEE, SWENDSEID M. E. & JACOB, R. A.: "Glutathione blood levels and other oxidant defense indices in men fed diets low in vitamin C". *J. Nutr.* 1991; 121:1.969-1.975.

HORNIG, D., WEBER, F. & WISS, O.: "Studies on the uptake of (1-14C)-ascorbic acid and (1-14C)-dehydroascorbic acid by platelets of guinea pigs". *Clin. Chem. Acta.* 1971;33: 187-196.

JACOB, R. A., SKALA, J. H. & OMAYE, S. T.: "Biochemical indices of vitamin C status". *Am. J. Clin. Nutr.* 1987; 46: 818-826.

JOHNSTON, C. S., MEYER, C. G. & SRILAKSHMI, J. C.: "Vitamin C elevates red blood cell glutathione in healthy adults". *Am. J. Clin. Nutr.* 1993; 58: 103-105.

KINSCHERF, R., FISCHBACH, T., MIHM, S., ROTH, S., HOHENHAUS–SIEVERT, E., WEISS, C., EDLER, L., BÄRTSCH, P. & DRÖGE, W.: "Effect of glutathione depletion and oral N–acetyl–cysteine treatment on $CD4^+$ and $CD8^+$ cells". *FASEB J.* 1994; 8:448–451

LEVINE, M.: "New concepts in the biology and biochemistry of ascorbic acid". *New Engl. J. Med.* 1986; 314:892-902.

MACHLIN, L. J.: "Beyond Deficiency. New views on the function and Health Effects of Vitamins". Introduction *Ann N. Y. Acad. Sci.* 1992; 669:1-6

MÅRTENSSON, J. & MEISTER, A.: "Glutathione deficiency decreases tissue ascorbate levels in newborn rats: Ascorbate spares glutathione and protects". *Proc. Natl. Acad. Sci.* 1991; 88: 4.656-4.660.

— "Glutathione deficiency increases hepatic acid synthesis in adult mice". *Proc. Natl. Acad. Sci.* 1992; 89: 11566-11568.

MEISTER, A.: "New aspects of glutathione biochemistry and transport: selective alteration of glutathione metabolism". *Nutr. Rev.* 1984; 43: 3.031-3.042.

— "Metabolism and function of glutathione". In: *Glutathione: Chemical, Biochemical and Medical Aspects-Part A.* (Ed: DOLPIN, D., POULSO, R. & AVRAMOVIC, O.), 1989; pp. 368-474. John Wiley & Sons, Inc.

— "Glutathione deficiency produced by inhibition of its synthesis and its reversal; applications in research and therapy". *Pharmac. Ther.* 1991;51: 155-194.

— "On the antioxidant effects of ascorbic acid and glutathione". *Biochem. Pharm.* 1992; 44: 1.905-1.915.

— "Glutathione–ascorbic acid antioxidant system in animals". *J. Biol. Chem.* 1994; 269:9.397–9.400.

MILBY, K., OKE, A. S., ADAMS, R. N.: "The detail mapping of ascorbate distribution in rat brain". *Neurosci Lett.* 1982: 28: 223-226.

MILLER, D. D. & KAPSOKEFALOU, M.: "Megadoses of ascorbic acid promote lipid oxidation in the serum of iron loaded guinea pigs". *FASEB J.* 1994; 8: Abstract n.º 4.135.

MITCHELL, J. R., THORGEIRSSON, S. S., POTTER, W. Z., JOLLOW, D. J., & KEISER, H.: "Acetaminophen-induced hepatic injury: protective role of glutathione in man and rationale for therapy". *Clin. Pharmac. Ther.* 1974;16:676-684.

PHILBERT, M. A., BEISWANGER, C. M., WATERS, D. K., REUHL, K. R., LOWNDES, H. E.: "Cellular and regional distribution of reduced glutathione in the nervous, system of the rat: Histochemical localization by mercury orange and o-phthaldialdehyde-induced histofluorescence". *Toxico Appl. Pharmacol.* 1991;107: 215-227.

RAPS, S. P., LAI, J. C., HERTZ, L., COOPER, A. J.: "Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons". *Brain Res.* 1989; 493: 398-401.

ROBERTSON, J. M., DONNER, A. P., TREVITHICK, J. R.: "A possible role for vitamins C and E in cataract prevention". *Am. J. Clin. Nutr.* 1991; 53: 346S-351S.

ROSE, R. C.: "Cerebral metabolism of oxidized ascorbate". *Brain Res.* 1993; 628:49-55.

ROSE, R. C. & BODE, A. M.: "Biology of free radicals scavengers: an evaluation of ascorbate". *FASEB J.* 1993;7:1.135-1.142.

SASTRE, J., PALLARDÓ, F. V., LLOPIS, J., FURUKAWA, T., VIÑA, J. R. & VIÑA, J.: "Glutathione depletion by hyperphagia-induced obesity". *Life Sci.* 1989;45:183-187.

SPECTOR, R.: "Penetration of ascorbic acid from cerebrospinal fluid into brain". *Exp. Neurol.* 1981; 72: 645-653.

SZENT-GYORGYI, A.: "Observations on the function of peroxidase systems and the chemistrym of the adrenal cortex". *Biochem J.* 1928; 22:1.387-1.409.

SUBCOMMITTEE ON THE TENTH EDITION OF THE RDAS FOOD AND NUTRITION BOARD: "Recommended Dietary Allowances". National Academy Press, 1989, Washington. *Chapter* 8: 115-124.

VERA, J. C., RIVAS, C. I., FISCHBARG, J., GOLDE, D. W.: "Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic–acid". *Nature.* 1993 ; 364: 79–82.

VIÑA, J. R., PALACÍN, M., PUERTES, I. R., & VIÑA, J.: "Role of the ∂-glutamyl cycle in the regulation of amino acid tranlocation". *Am. J. Physiol.* 1989 a; 257 (Endocrinol. Metab. 20): E916-E922.

VIÑA, J. R., DEVESA, A., GARCÍA, C., PUERTES, O'CONNOR, E.: "Maintenecae of GSH in primary astrocyte cultures under oxidative stress conditions". *Biochem. Soc. Trans.* 1992; 21: 74S

VIÑA, J. R., SAEZ, G. T., VIÑA, J.: "The physiological functions of glutathione". In: *CRC Handbook of Biochemistry of Free Radicals and Antioxidants* (Ed: MIQUEL, J., QUINTANILLA, A. T., WEBER, H.), 1989 b, vol. 1 pp 81-94. CRC Press, Boca Raton, Florida.

WELLS, W. W., XU, P., YANG, Y. & ROCQUE, A.: "Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity". *J. Biol. Chem.* 1990; 265: 15.361-15.364.

WILSON, J. X.: "Ascorbic acid uptake by a high-affinity sodium-dependent mechanism in cultured rat astrocytes". *J. Neurochem.* 1989; 53: 1.064-1.071.

WILSON, J. D.: "Vitamin deficiency and excess". In: *Harrison's Principles of Internal Medicine* (Eds: ISSELBACHER, K. J., BRAUNWALD, E., WILSON, J. D., MARTIN, J. B., FAUCCI, A. S. & KASPER, D. L.). McGraw-Hill Inc, New York, 1994, pp. 472-480.

Table I.–Standard reduction potentials ($\varepsilon^{o'}$) of some Biochemical half-reactions

Half–reaction	$\varepsilon^{o'}$ *(mV)*
$HO\cdot$, H^+ /H_2O	2310
$O3\cdot^-$, $2H^+$ / H_2O + O_2	1800
$RO\cdot$, H^+ / ROH	1600
$HOO\cdot$, H^+ /H_2O_2	1060
$ROO\cdot$, H^+ / ROOH	≈1000
$O_2\cdot^-$, 2 H^+ /H_2O_2	940
$O_2/O_2\cdot^-$	650
PUFA$\cdot$, H^+ / PUFA–H	600
α–Tocopheroxyl$\cdot$, H^+ / α–Tocopherol (vitamin E)	500
H_2O_2, H^+ /H_2O, $HO\cdot$	320
Ascorbate$\cdot^-$, H^+ /Ascorbate monoanion (vitamin C)	282
Semiubiquinone, H^+ / Ubiquinol	200
Ubiquinone, H^+ / Semiubiquinone	–36
Dehydroascorbic / Ascorbate$\cdot^-$	–174
O_2 / $O_2\cdot^-$	–330
O_2, H^+ /$HO_2\cdot$	–460
GSSG / GSSG$\cdot^-$	–1500
H_2O / e^-_{aq}	–2870

All the values have been taken from Buettner (1993).

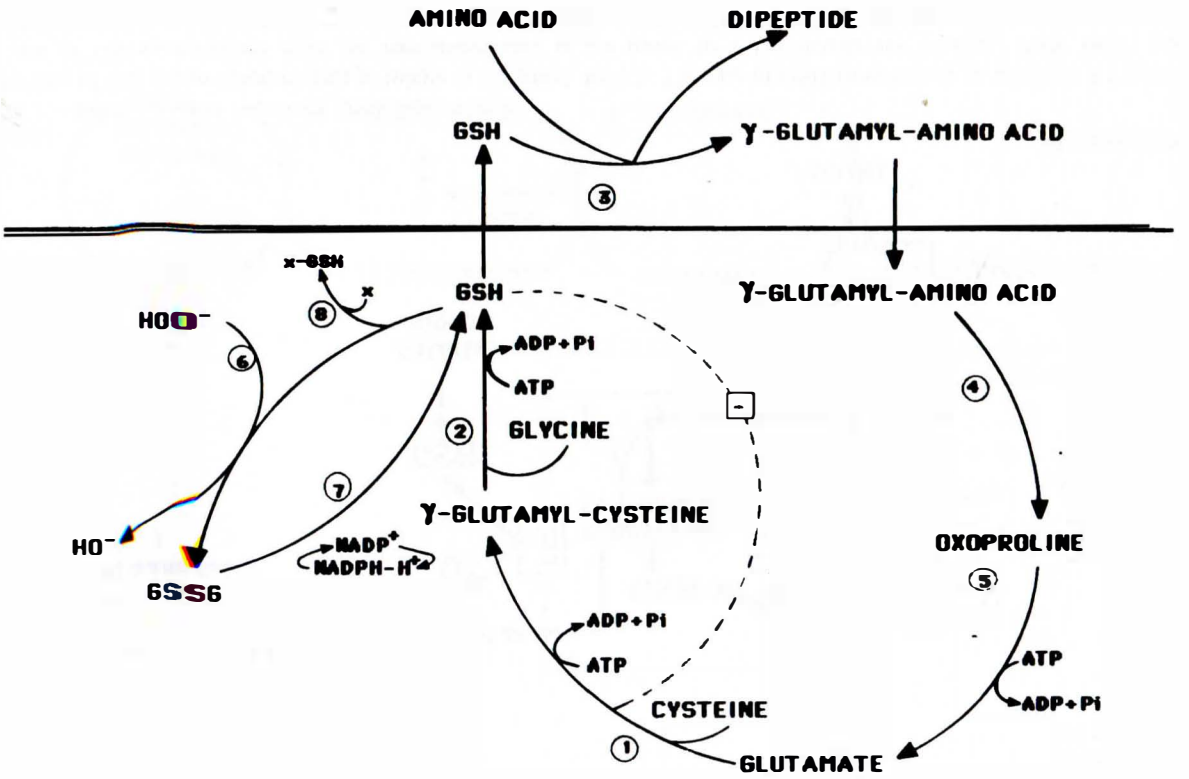

Figure 1.—The gamma–glutamyl cycle.
1: ∂–glutamyl–cysteine synthetase; 2: GSH–synthetase; 3: ∂–glutamyltranspeptidase is a membrane–bound enzyme that starts the degradation of GSH. This reaction takes place outside the cell; 4: ∂–glutamylcyclotransferase; 5: 5–oxoprolinase. 6: GSH–peroxidase; 7: GSSG–reductase; 8: GSH–transferase.

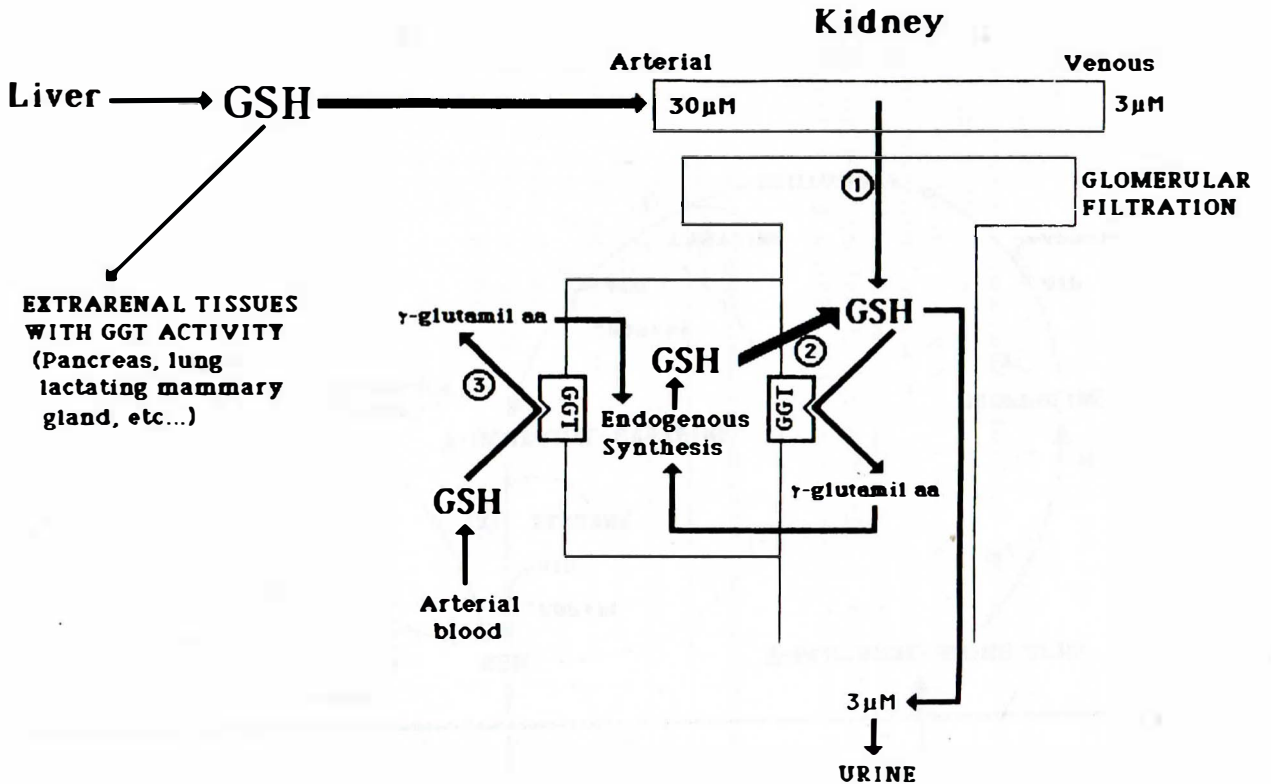

Figure 2.—Intertissue and intrarenal GSH flux.
1: The rate of GSH from blood plasma to tubule is 0.8 µmol. hr^{-1}; 2: The rate of GSH from kidney to tubule is 4.1 µmol. hr^{-1}; The rate of GSH metabolism from the basolateral side is 2.4 µmol. hr^{-1}. For details see Meister, 1989. GGT: gamma–glutamyltranspeptidase; ∂–glutamyl aa: gamma–glutamyl amino acids; GSH: reduced glutathione.

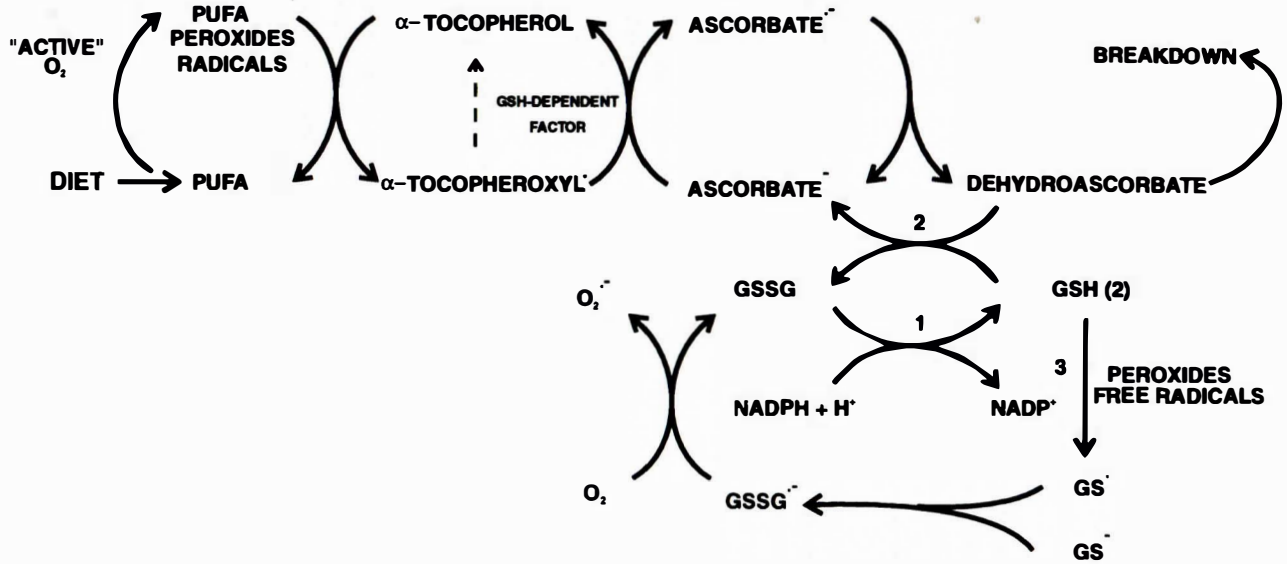

Figure 3.—Interrelationship between ascorbate and glutathione.
1: Glutathione disulfide reductase; 2: Transhydrogenases; 3: Glutathione peroxidase.
Abbreviations used: Ascorbate⁻ → Ascorbate monoanion; Ascorbate·⁻ → semidehydroascorbate radical (ascorbate free radical); GSH → glutathione; GSSG → glutathione disulfide; GS· → glutathiyl radical; PUFA → polyunsaturated fatty acids; tocopheroxyl · → tocopherol free radical.