Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4656–4660, June 1991
Biochemistry

Glutathione deficiency decreases tissue ascorbate levels in newborn rats: Ascorbate spares glutathione and protects

(buthionine sulfoximine/mitochondrial damage/hydrogen peroxide/cataracts)

JOHANNES MRTENSSON AND ALTON MEISTER

Department of Biochemistry, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

Contributed by Alton Meister, March 8, 1991

ABSTRACT **Glutathione deficiency in newborn rats, produced by administration of L-buthionine-(*S,R*)-sulfoximine, a transition-state inactivator of γ-glutamylcysteine synthetase, decreases ascorbate levels of kidney, liver, brain, and lung. These tissues, especially their mitochondria, undergo severe damage and the animals die within a few days. When glutathione levels are markedly decreased, ascorbate levels decrease leading to formation of dehydroascorbate, which is degraded. Ascorbate has high antioxidant activity, but it (and other antioxidants such as α-tocopherol) must be maintained in reduced forms. These studies show *in vivo* that an important function of glutathione is to maintain tissue ascorbate. Administration of large doses of ascorbate (but not of dehydroascorbate) to buthionine sulfoximine-treated newborn rats decreases mortality, leads to normal levels of ascorbate, and spares glutathione. Newborn rats given lower doses of buthionine sulfoximine develop cataracts that, as shown previously, can be prevented by giving glutathione monoester; as found here, such cataracts can be partially prevented by administration of high doses of ascorbate or dehydroascorbate. Ascorbate spares glutathione indicating that these compounds have similar antioxidant actions. Ascorbate may have reductive functions that are not efficiently performed by glutathione. Although glutathione normally functions to maintain ascorbate, α-tocopherol, and other cellular components in reduced states, ascorbate can serve as an essential antioxidant in the presence of severe glutathione deficiency.**

Glutathione (GSH) provides cells with their reducing milieu and protects them against the toxic effects of hydrogen peroxide and other reactive oxygen species (1–3). Inhibition of GSH synthesis by administration to mice of L-buthionine-(*S,R*)-sulfoximine (BSO), a transition-state inactivator of γ-glutamylcysteine synthetase (4–7), leads to markedly decreased GSH tissue levels (8) and to damage to skeletal muscle (9), lung type 2 cells (10), and epithelial cells of the jejunum and colon (11). Treatment of newborn mice (12, 13) and rats (13) with moderate doses of BSO leads to cataracts; in larger doses, BSO produces brain damage (14) and death (13, 14). Tissue damage associated with GSH deficiency is invariably accompanied by mitochondrial degeneration, which is prevented (9–11, 13, 14) by giving GSH monoesters (15–18). Mitochondria, which obtain GSH by transport from the cytosol (19, 20), produce significant amounts of hydrogen peroxide (21–24) whose accumulation in GSH deficiency leads to mitochondrial and other types of cell damage. Cellular damage associated with GSH deficiency is produced by products formed in normal cellular metabolism, which may not be equivalent to those formed in systems in which external stress (e.g., increased oxygen, drugs, or radiation) is applied.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Table 1. Mortality in newborn rats produced by BSO-induced GSH deficiency is decreased by ascorbate but not by dehydroascorbate

Exp.	Treatment	Mortality: No. died/ total no.	Mortality: %
1	None (controls)	0/30	0
2	BSO	43/47	91
3	BSO + ascorbate (2 mmol/kg)	5/45	11
4	BSO + ascorbate (0.4 mmol/kg)	4/10*	40
5	BSO + ascorbate (0.2 mmol/kg)	8/10	80
6	BSO + ascorbate (0.005 mmol/kg)	10/10	100
7	BSO + dehydroascorbate (2 mmol/kg)	18/18	100

Newborn rats were injected i.p. with BSO (3 mmol/kg; isosmolar solution) twice daily (at 0900 and 2100) on days 2–5 of life starting 24–28 hr after birth. Ascorbate and dehydroascorbate (1 mmol/kg; isosmolar, pH 6.8–7.0) were injected i.p. twice daily (at 1100 and 2300). Equivalent volumes, made up with saline, were given to all animals. Deaths occurred between days 4 and 6 of life. Rats given dehydroascorbate died on days 3 or 4. The weights of rats (mean ± SD; day 5) were 9.80 ± 0.45 g (controls), 7.80 ± 0.45 g (treated with BSO), 9.70 ± 0.27 g (treated with BSO plus ascorbate at 2 mmol/kg/day), and 9.30 ± 0.27 g (treated with BSO and ascorbate at 0.4 mmol per kg per day). Cataracts developed in all of the surviving BSO-treated rats; five had unilateral cataracts.
*Statistically different ($P < 0.05$) from experiment 3.

GSH functions directly in the destruction of hydrogen peroxide and lipid peroxides by providing a substrate for the GSH peroxidases (25–28). GSH also functions as an antioxidant by promoting formation of the reduced forms of other antioxidants such as ascorbate and α-tocopherol (29, 30). It has long been thought that GSH reacts with dehydroascorbate to form ascorbate (31–36). Availability of an experimental model in which GSH synthesis can be selectively inhibited has increased understanding of cellular GSH-dependent reactions and facilitates study of the specificity and other aspects of GSH function (3, 8, 37).

We have used newborn rats, which are highly sensitive to GSH deficiency (13, 14). Depletion of GSH led to depletion of tissue ascorbate. Treatment of GSH-deficient rats with high doses of ascorbate decreased mortality indicating that ascorbate can itself serve an essential antioxidant function (38).

EXPERIMENTAL PROCEDURES

Newborn rats (Sprague–Dawley, in litters of 10–12, both sexes) were breast fed ad libitum. BSO (4–7) was crystallized from 80% (vol/vol) ethanol in 90% yield. Ascorbic and dehydroascorbic acids were obtained from Sigma. The purity

Abbreviations: GSH, glutathione; BSO, L-buthionine-(*S,R*)-sulfoximine.

Table 2. GSH levels of tissues of newborn rats treated with BSO and ascorbate

Tissue	GSH, μmol/g			
	Control	BSO	BSO + ascorbate (2 mmol/kg)	BSO + ascorbate (0.4 mmol/kg)
Kidney	2.50 ± 0.25	0.043 ± 0.009*	0.094 ± 0.018†	0.092 ± 0.013†
Liver	3.80 ± 0.20	0.017 ± 0.009*	0.044 ± 0.009†	0.049 ± 0.005†
Lung	1.35 ± 0.08	0.040 ± 0.017*	0.14 ± 0.02†	0.12 ± 0.01†
Brain	1.20 ± 0.30	0.092 ± 0.030*	0.14 ± 0.01†	0.14 ± 0.01†

Tissue levels of GSH (expressed as mean ± SD; $n = 4$) were determined on samples obtained at 1300 (2 hr after injection of ascorbate or saline) on day 5. Injections of BSO, ascorbate, and dehydroascorbate were performed as stated in Table 1. Brain refers to cerebral cortex. Statistically significantly different data ($P < 0.05$) with respect to control (*) and BSO (†) groups are indicated.

of the ascorbic acid was >90%, determined as described (39, 40). Isosmolar solutions of sodium ascorbate and dehydroascorbate were prepared immediately prior to use in ice-cold sterile glass-distilled water saturated with N_2 and adjusted to pH 6.8–7.0 by cautious addition of 2 M NaOH.

The tissues were obtained essentially as described (10); homogenization was done (within 1 min) in sulfosalicylic acid containing 3 mM Na_2EDTA. Mitochondrial fractions were obtained as described (9–11, 13). Ascorbate (39) and total ascorbate (41) were determined on the same samples used for determinations of GSH (42). Ascorbate was stable in the samples prepared under these conditions; thus, they could be stored at −70°C for 24 hr with >98% recovery. Tissues were prepared for microscopy as stated (10, 13). For light microscopy, they were embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Statistical analyses were performed by the one-way analysis of variance and followed by the Tukey test for multiple comparison. The incidence of cataracts and mortality was evaluated by the t test for independent samples.

RESULTS

Protection by Ascorbate Against Mortality Due to GSH Deficiency. GSH deficiency was induced in newborn rats (24–28 hr of age) by giving BSO (3 mmol/kg, twice daily); about 90% of the rats died within 6 days of life (Table 1). Administration of BSO led to markedly decreased tissue levels of GSH (Table 2) and of ascorbate (Table 3). Light microscopy and electron microscopy revealed damage in kidney, liver, lung, and brain. Striking mitochondrial swelling and degeneration were observed in the cerebral cortex (14) and in the other tissues (J.M., A. Jain, E. Stole, P. Auld, W. Frayer, and A.M., unpublished data). In kidney there was proximal tubular microvillus degeneration and margination of nuclear chromatin and cell necrosis (Fig. 1*B*). The liver showed central pallor and decreased extramedullary hematopoesis with reduced numbers of erythroblasts and megakaryocytes (Fig. 1*D*). The lung showed decreased numbers of lamellar bodies in type 2 cells, capillary endothelial swelling, and decreased amounts of intraalveolar tubular myelin. The heart showed no significant changes. [GSH deficiency produced in adult mice by administration of BSO for 1–3 weeks led to lung type 2 cell degeneration, but no damage was found by electron microscopy in liver or kidney (10).]

Mortality was significantly decreased when ascorbate (two daily doses of 1 mmol/kg) was given with BSO (Table 1), and the structural changes described above were not found except for reduced height of renal proximal tubular microvilli. Lower doses of ascorbate were less or not protective. Dehydroascorbate did not protect and appeared to hasten death.

Effects of GSH Deficiency on Ascorbate Levels and of Treatment with Ascorbate on GSH and Ascorbate Levels. Administration of BSO led to the expected marked decreases in the GSH levels of kidney, liver, lung, and brain (Table 2). When ascorbate (2 mmol per kg per day) was given with BSO, the GSH levels were significantly greater than when only BSO was given. A lower dose of ascorbate (0.4 mmol per kg per day) gave essentially the same results. Mitochondrial GSH levels were also greatly decreased after treatment with BSO (Table 4), and they were significantly increased (much more so than the corresponding total tissue levels) by administration of ascorbate. The levels of GSH in heart mitochondria decreased only ≈50% after treatment with BSO and were not increased by treatment with ascorbate.

Depletion of GSH (Table 2) is accompanied by depletion of ascorbate and total ascorbate (Table 3); the differences between the values for total ascorbate and those for ascorbate, which are a measure of dehydroascorbate, were substantially greater after treatment with BSO than in the controls or in animals treated with BSO and ascorbate. Ascorbate and total ascorbate values were much lower at birth than after 5 days of life, indicating that ascorbate synthesis is initially very low, that it is rapidly utilized, or both.

Table 3. GSH deficiency decreases tissue ascorbate levels

Tissue	Ascorbate, μmol/g			
	Control	BSO	BSO + ascorbate (2 mmol/kg)	BSO + ascorbate (0.4 mmol/kg)
Kidney	2.58 ± 0.13	0.58 ± 0.16*	2.58 ± 0.24†	2.69 ± 0.21†
	[2.72 ± 0.13]	[1.36 ± 0.27]*	[2.66 ± 0.47]	[2.63 ± 0.30]
Liver	2.51 ± 0.30	0.42 ± 0.15*	3.08 ± 0.50†	2.76 ± 0.28†
	[2.60 ± 0.28]	[1.14 ± 0.23]*	[3.13 ± 0.43]†	[2.80 ± 0.44]†
Lung	2.46 ± 0.30	0.70 ± 0.32*	2.74 ± 0.42†	2.93 ± 0.46†
	[2.56 ± 0.25]	[1.52 ± 0.16]*	[3.12 ± 0.74]†	[2.90 ± 0.90]†
Brain	5.81 ± 0.44	2.71 ± 0.22*	4.86 ± 0.17†	4.77 ± 0.35*†
	[5.98 ± 0.29]	[4.02 ± 0.85]*	[4.75 ± 0.70]	[5.31 ± 0.98]

Tissue levels (values are mean ± SD; $n = 4$) of ascorbate and "total" ascorbate (given in brackets) were determined on samples obtained at 1300 (2 hr after injection of ascorbate or saline) on day 5 (after given seven doses of BSO over 3.5 days). The ascorbate [total ascorbate] values after two doses of BSO (14 hr) for kidney, liver, lung, and cerebral cortex (brain), were, respectively, 0.40 [0.50], 0.13 [0.29], 0.04 [0.46], and 2.00 [2.83] μmol/g. The data for the controls at this age are, respectively, 0.72 [1.01], 0.65 [0.94], 0.60 [0.94], and 1.99 [2.79]. Statistically significantly different data ($P < 0.025$) with respect to controls (*) and BSO (†) groups are indicated.

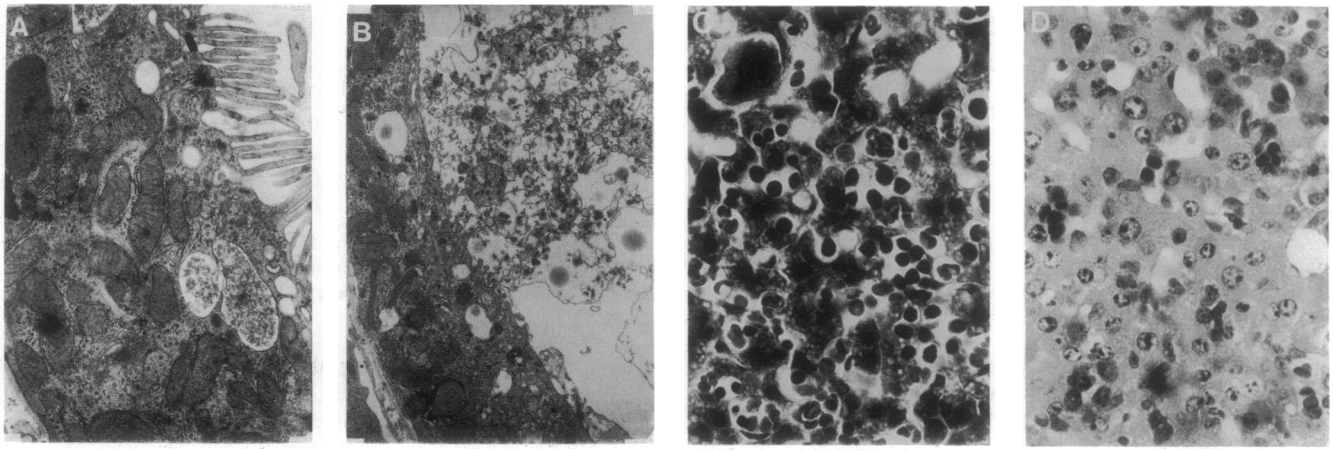

FIG. 1. (*A* and *B*) Electron microscopy of kidney. (*A*) Control. (×8400.) (*B*) BSO-treated rat. (×6000.) (*C* and *D*) Light microscopy of liver. (*C*) Control. (*D*) BSO-treated rat. (×650.)

Prevention of Cataracts by Ascorbate and Dehydroascorbate. Cataract formation was examined in newborn rats using the model described (13); rats were given BSO (3.0 mmol/kg) at 36 and 60 hr of age, and the lenses were examined at 14–15 days of age when the rats opened their eyes. Almost all of the BSO-treated rats had cataracts (Table 5). When ascorbate (2 mmol per kg per day) was given with BSO, the incidence of cataracts decreased. Administration of dehydroascorbate was also protective suggesting that dehydroascorbate is reduced to ascorbate under these conditions. Lens mitochondrial GSH decreased after administration of BSO in this model, and a higher GSH level was found when ascorbate was given with BSO (Table 4). The total level of lens GSH decreased from control values of 5.6 ± 0.25 μmol/g to 0.30 ± 0.02 μmol/g. A value of 0.40 ± 0.02 μmol/g was found after giving BSO plus ascorbate; for treatment with BSO plus dehydroascorbate the value was 0.32 ± 0.02 μmol/g. Treatment with BSO led to decreased levels of ascorbate and total ascorbate in lens and eye, and increases in these values were found after treatment with BSO plus ascorbate and with BSO plus dehydroascorbate (Table 6). (The values for lens ascorbate may be unduly low due to oxidation during dissection of the lens, which takes several minutes.) The ascorbate and total ascorbate values in the livers of these rats were substantially greater after giving BSO and dehydroascorbate than after giving BSO alone.

Other Findings. Treatment of adult mice led to decreased weight gain; this was prevented by administration of GSH ester (10, 11). The weights of newborn rats treated with BSO were decreased 14–20% as compared with controls and BSO-plus ascorbate-treated rats (Tables 1 and 5). BSO treatment increased serum levels of creatinine (to 0.45 ± 0.05 mg/100 ml; $n = 4$; controls, 0.22 ± 0.05 mg/100 ml; BSO-plus ascorbate-treated rats, 0.25 ± 0.05 mg/100 ml), and urea (to 48 ± 7 mg/100 ml; $n = 4$; controls, 22 ± 4 mg/100 ml; BSO-plus ascorbate-treated rats, 18 ± 1 mg/100 ml). GSH deficiency in newborn rats led to significant increases in the plasma levels of triglycerides (≈250%) and cholesterol (≈50%) and a decrease of the level of reticulocytes (3% of controls); these changes were not found after giving BSO plus ascorbate. Other effects of GSH deficiency will be described elsewhere (J.M., A. Jain, E. Stole, P. Auld, W. Frayer, and A.M., unpublished data).

Table 4. Mitochondrial GSH levels in tissues of newborn rats treated with BSO and ascorbate

Tissue	GSH, nmol/mg of protein		
	Control	BSO	BSO + ascorbate
Kidney	8.29 ± 0.70	0.20 ± 0.03*	0.85 ± 0.13†
Liver	5.50 ± 0.10	0.31 ± 0.02*	0.83 ± 0.20†
Lung	7.63 ± 0.21	0.27 ± 0.06*	1.61 ± 0.09*†
Brain	9.17 ± 0.38	0.91 ± 0.05*	2.49 ± 0.08*†
Heart	7.40 ± 0.70	3.43 ± 0.31*	2.40 ± 0.13*†
Lens	11.0 ± 0.17	2.30 ± 0.30*	3.60 ± 0.35*†

BSO and ascorbate were given as in Table 1. Mitochondrial GSH (expressed as mean ± SD; $n = 3$–5) was determined at 1300 on day 5, 2 hr after administration (see Table 1) of saline or ascorbate (1 mmol/kg). For lens, BSO was given in two doses of 3.0 mmol/kg at 36 and 60 hr of age. Statistically significant data ($P < 0.05$) with respect to control (*) or BSO (†) groups are indicated.

DISCUSSION

GSH acts as an antioxidant by serving as substrate of several enzymes that reduce hydrogen peroxide and organic peroxides (25–28, 43, 44) and by mediating the reduction of dehydroascorbate (29, 31–36, 45–48) and the oxidized forms of α-tocopherol and other compounds (30, 49–57). Thus, GSH deficiency would be expected to produce effects that also result from deficiency of ascorbate and α-tocopherol. GSH deficiency decreases tissue ascorbate; that total ascorbate levels also decrease may be ascribed to metabolic degradation of dehydroascorbate (34, 58). Since α-tocopherol is maintained in reduced form by GSH (or ascorbate), the effect of GSH deficiency on α-tocopherol levels should be examined directly.

Ascorbate protects against GSH deficiency in two newborn rat models; thus, ascorbate (2 mmol/day) protects

Table 5. Cataracts formed in newborn rats by BSO-induced GSH deficiency are partially prevented by ascorbate and dehydroascorbate

Exp.	Treatment	Cataracts (bilateral)	
		No. cataracts/no. rats	%
1	None (controls)	0/30	0
2	BSO	37/38	97
3	BSO + ascorbate (2 mmol/kg)	3/32	9
4	BSO + dehydroascorbate (2 mmol/kg)	3/14	21

Newborn rats were injected intraperitoneally with BSO (3.0 mmol/kg) at 36 and 60 hr of age. Ascorbate and dehydroascorbate were given as in Table 1. The weights of the rats (mean ± SD, day 14) were, respectively, 38.6 ± 0.88 g (controls, $n = 9$), 33.4 ± 1.9 g (treated with BSO), 38.2 ± 0.63 g (treated with BSO and ascorbate at 2 mmol per kg per day), and 36.1 ± 0.83 g (treated with BSO and dehydroascorbate at 2 mmol per kg per day). Experiments 3 and 4 are not statistically different.

Table 6. Ascorbate levels in lens and eye

Treatment	Ascorbate, μmol/g		
	Lens	Eye	Liver
Untreated	0.47 ± 0.08	1.83 ± 0.16	2.51 ± 0.37
	[0.78 ± 0.09]	[1.80 ± 0.13]	[2.60 ± 0.12]
BSO	0.03 ± 0.03*	1.01 ± 0.11*	0.79 ± 0.10*
	[0.50 ± 0.02]	[1.25 ± 0.40]*	[1.25 ± 0.40]*
BSO + ascorbate	0.26 ± 0.04*†		
	[1.24 ± 0.06]*†		
BSO + dehydro-ascorbate	0.12 ± 0.06*†	1.75 ± 0.22†	1.66 ± 0.19*†
	[1.17 ± 0.21]†	[2.01 ± 0.09]†	[2.11 ± 0.21]*†

Ascorbate and total ascorbate (in brackets) levels were determined (mean ± SD; $n = 4$) on samples obtained from the rats studied in Table 5. Statistically significant data ($P < 0.05$) with respect to control (*) or BSO (†) groups are indicated.

against mortality associated with GSH deficiency and also against cataracts formed in a model in which GSH deficiency in the lens is produced by giving a lower dose of BSO. Notably dehydroascorbate did not protect against mortality but did protect in the cataract model, in which there is less severe GSH depletion that allows conversion of dehydroascorbate to ascorbate. High levels of ascorbate are found in the lens, an observation made >50 years ago (59); it has often been suggested that deficiency of ascorbate and GSH is related to formation of cataracts (see, for example, refs. 13 and 59–62).

GSH-dependent reduction of dehydroascorbate to ascorbate is catalyzed by mammalian glutaredoxin and protein disulfide isomerase (63). The present studies provide strong evidence that this reaction takes place *in vivo*. The marked decrease in ascorbate and increase of dehydroascorbate produced by GSH depletion (Table 3) is impressive and indicates that the reduction of dehydroascorbate is an important physiological function of GSH. This is supported by the finding that treatment of GSH-deficient newborn rats with large doses of ascorbate leads to significant sparing of GSH,

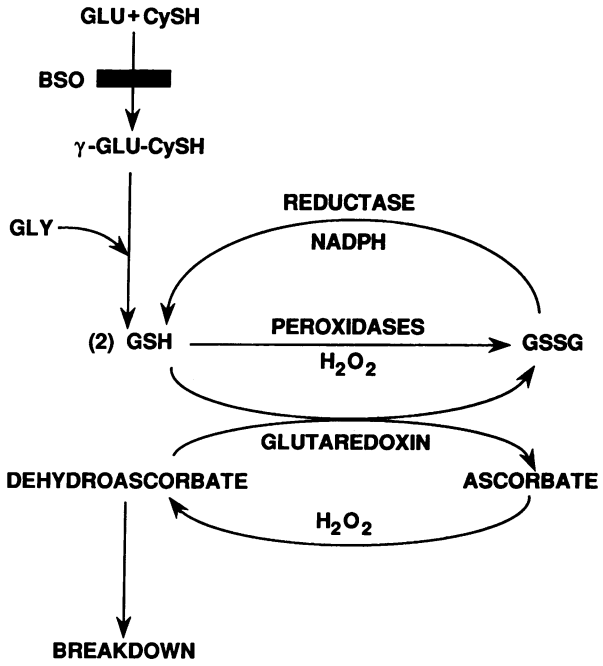

FIG. 2. Ascorbate and GSH act to destroy peroxide (and related active oxygen forms). Glutaredoxin and protein disulfide isomerase (63) catalyze GSH-dependent reduction of dehydroascorbate. GSSG, glutathione disulfide.

which is especially notable in the mitochondria (Table 4), a major source of cellular hydrogen peroxide (21–24). By sparing GSH, ascorbate facilitates its own recycling as well as promoting other reactions that require GSH.

The findings show that GSH and ascorbate have actions in common in the destruction of reactive oxygen species (Fig. 2). GSH reacts with reactive oxygen compounds and it functions in the reduction of hydrogen peroxide and organic peroxides in reactions catalyzed by the GSH peroxidases (selenium-containing and other). It is well known that ascorbate can also interact with peroxides and other reactive oxygen forms (29). Such reactions occur nonenzymatically, but interaction of hydrogen peroxide and ascorbate is enzyme-catalyzed in chloroplasts, cyanobacteria, and soybean nodules (64), and an analogous reaction may occur in mammalian tissues. It is possible that ascorbate reduces compounds (e.g., α-tocopherol) that are not directly reduced by GSH. Export of GSH, a property of many cells, appears to serve as part of a recycling mechanism that protects the cell membrane against oxidative damage by maintaining essential thiols and other membrane components such as α-tocopherol; this pathway has been reviewed (65).

That ascorbate protects against experimental GSH deficiency suggests that it may be useful in treating patients with inborn deficiencies of γ-glutamylcysteine and GSH synthetases (66), premature newborns, and others deficient in GSH (67–69). Although ascorbate can induce oxidative stress (see ref. 70), the dose used here (2 mmol per kg per day) was highly effective in preventing mortality induced by severe GSH deficiency. However, we found that doses of 5 mmol per kg per day are fatal for newborn rats.

The biochemical basis of the mortality observed here is GSH deficiency and associated oxidative effects. Notably, guinea pigs are also highly sensitive to GSH deficiency and die after receiving several doses of BSO (71); mortality is reduced by administration of high doses of ascorbate (ref. 72 and J. Han, O. W. Griffith, and J.M., unpublished data). Rats and guinea pigs differ in that the former, but not the latter, can synthesize ascorbate. Newborn rats are much more sensitive than adult rats (and mice) to GSH deficiency and their dependence on GSH seems to be similar to that of guinea pigs. We have noted that BSO is retained to a greater extent by the tissues of newborn rats as compared to adults.* This may partially explain the high sensitivity of newborns to BSO-induced GSH deficiency.

*For example, 3 days after a single injection of BSO (2 mmol/kg) to newborn rats, the BSO levels of liver and lung were still significant (i.e., 30 μM and 13 μM, respectively); in contrast, BSO levels decreased to 18 μM and 35 μM in these tissues of adult mice only 6 hr after BSO injection (5 mmol/kg) and thereafter decreased further.

We thank Dr. D. A. Fischman and Mrs. L. Cohen-Gould for assistance in the electron microscopy studies and Drs. H. T. Nguyen and T. Godwin for kind help with the light microscopy studies. K. Lamhour provided skillful technical assistance. The research was supported in part by a grant to A.M. from the Public Health Service, National Institutes of Health (R37 DK-12034). J.M. acknowledges stipendary support from the AGA AB Medical Research Fund and the Draco Medical Research fund.

1. Dolphin, D., Poulson, R. & Avramovic, O., eds. (1989) *Glutathione Chemical, Biochemical, and Medical Aspects* (Wiley, New York), Parts A & B.
2. Larsson, A., Orrenius, S., Holmgren, A. & Mannervik, B., eds. (1983) *Functions of Glutathione Biochemical, Physiological, Toxicological, and Clinical Aspects* (Raven, New York).
3. Meister, A. & Anderson, M. E. (1983) *Annu. Rev. Biochem.* **52**, 711–760.
4. Griffith, O. W., Anderson, M. E. & Meister, A. (1979) *J. Biol. Chem.* **254**, 1205–1210.

5. Griffith, O. W. & Meister, A. (1979) *J. Biol. Chem.* **254,** 7558–7560.
6. Meister, A. (1978) in *Enzyme-Activated Irreversible Inhibitors*, eds. Seiler, N., Jung, M. J. & Koch-Weser, J. (Elsevier–North Holland, Amsterdam), pp. 187–211.
7. Griffith, O. W. (1982) *J. Biol. Chem.* **257,** 13704–13712.
8. Griffith, O. W. & Meister, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6319–6322.
9. Mårtensson, J. & Meister, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 571–475.
10. Mårtensson, J., Jain, A., Frayer, W. & Meister, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5296–5300.
11. Mårtensson, J., Jain, A. & Meister, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1715–1719.
12. Calvin, H. I., Medvedovsky, C. & Worgul, B. V. (1986) *Science* **233,** 553–555.
13. Mårtensson, J., Steinherz, R., Jain, A. & Meister, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8727–8731.
14. Jain, A., Mårtensson, J., Stole, E., Auld, P. A. M. & Meister, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1913–1917.
15. Puri, R. N. & Meister, A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4714–4717.
16. Wellner, V. P., Anderson, M. E., Puri, R. N., Jensen, G. L. & Meister, A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4732–4735.
17. Anderson, M. E., Powrie, F., Puri, R. N. & Meister, A. (1985) *Arch. Biochem. Biophys.* **239,** 538–548.
18. Anderson, M. E. & Meister, A. (1989) *Anal. Biochem.* **183,** 16–20.
19. Griffith, O. W. & Meister, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4668–4672.
20. Mårtensson, J., Lai, J. C. K. & Meister, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7185–7189.
21. Loschen, G., Flohe, L. & Chance, B. (1971) *FEBS Lett.* **18,** 261–264.
22. Boveris, A., Oshino, N. & Chance, B. (1972) *Biochem. J.* **128,** 617–630.
23. Boveris, A. & Chance, B. (1973) *Biochem. J.* **134,** 707–716.
24. Forman, H. J. & Boveris, A. (1982) in *Free Radicals in Biology*, ed. Pryor, W. A. (Academic, New York), Vol. 5, pp. 65–90.
25. Mills, G. C. (1957) *J. Biol. Chem.* **229,** 189–197.
26. Flohe, L. (1989) in *Glutathione Chemical, Biochemical, and Medical Aspects*, eds. Dolphin, D., Poulson, R. & Avramovic, O. (Wiley, New York), Part A, pp. 643–731.
27. McCay, P. B. & Powell, S. R. (1989) in *Glutathione Chemical, Biochemical, and Medical Aspects*, eds. Dolphin, D., Poulson, R. & Avramovic, O. (Wiley, New York), Part B, pp. 111–151.
28. Mannervik, B. (1985) *Methods Enzymol.* **113,** 490–495.
29. Burns, J. J., Rivers, J. M. & Machlin, L. J., eds. (1987) *Ann. N.Y. Acad. Sci.* **498.**
30. Diplock, A. T., Machlin, L. J., Packer, L. & Pryor, W. A., eds. (1989) *Ann. N.Y. Acad. Sci.* **570.**
31. Szent-Györgyi, A. (1928) *Biochem. J.* **22,** 1387–1409.
32. Hopkins, F. G. & Morgan, E. J. (1936) *Biochem. J.* **30,** 1446–1462.
33. Crook, E. M. (1941) *Biochem. J.* **35,** 226–236.
34. Borsook, H., Davenport, H. W., Jeffreys, C. E. P. & Warner, R. C. (1937) *J. Biol. Chem.* **117,** 237–279.
35. Crook, E. M. & Hopkins, F. G. (1938) *Biochem. J.* **32,** 1356–1363.
36. Bigley, R., Stankova, L., Roos, D. & Loos, J. (1980) *Enzyme* **25,** 200–204.
37. Meister, A. (1989) in *Glutathione Centennial: Molecular Properties and Clinical Implications*, ed. Taniguchi, N. (Academic, New York), pp. 3–21.
38. Mårtensson, J. & Meister, A. (1991) *FASEB J.* **5,** A4710 (abstr.).
39. Omaye, S. T., Turnbull, J. D. & Sauberlich, H. E. (1979) *Methods Enzymol.* **62,** 3–7.
40. Dawson, R. M. C., Elliot, D. C., Elliot, W. H. & Jones, K. M., eds. (1969) *Data for Biomedical Research* (Oxford Univ. Press, New York), p. 437.
41. Roe, J. H. & Kuether, C. A. (1943) *J. Biol. Chem.* **147,** 399–407.
42. Anderson, M. E. (1985) *Methods Enzymol.* **113,** 548–555.
43. Burk, R. F., Trumble, M. J. & Lawrence, R. A. (1980) *Biochim. Biophys. Acta* **618,** 35–41.
44. Ursini, F., Maiorino, M., Valente, M., Ferri, L. & Gregolin, C. (1981) *Biochim. Biophys. Acta* **710,** 197–211.
45. Hendry, J. M., Easson, L. H. & Owen, J. A. (1964) *Clin. Chim. Acta* **9,** 498–499.
46. Bigley, R., Riddle, M., Layman, D. & Stankova, L. (1981) *Biochim. Biophys. Acta* **659,** 15–22.
47. Rose, R. C. (1989) *Am. J. Physiol.* **256,** F52–F56.
48. Christine, L., Thomson, G., Iggo, B., Brownie, A. C. & Stewart, C. P. (1956) *Clin. Chim. Acta* **1,** 557–569.
49. Packer, J. E., Slater, T. F. & Wilson, R. L. (1979) *Nature (London)* **278,** 747–748.
50. Niki, E., Tsuchiya, J., Tanimura, R. & Kamiya, Y. (1982) *Chem. Lett.*, 789–792.
51. Reddy, C. C., Scholz, R. W., Thomas, C. E. & Massaro, E. J. (1982) *Life Sci.* **31,** 571–576.
52. Doba, T., Burton, G. W. & Ingold, K. U. (1985) *Biochim. Biophys. Acta* **835,** 298–303.
53. Niki, E. (1987) *Ann. N.Y. Acad. Sci.* **498,** 186–199.
54. Leedle, R. A. & Aust, S. D. (1990) *Lipids* **25,** 241–245.
55. Graham, K. S., Reddy, C. C. & Scholz, R. W. (1989) *Lipids* **24,** 909–914.
56. Scholich, H., Murphy, M. E. & Sies, H. (1989) *Biochim. Biophys. Acta* **1001,** 256–261.
57. Wefers, H. & Sies, H. (1988) *Eur. J. Biochem.* **174,** 353–357.
58. Kagawa, Y., Mano, Y. & Shimazono, N. (1960) *Biochim. Biophys. Acta* **43,** 349–351.
59. von Euler, H. & Martius, C. (1933) *Hoppe-Seyler's Zeit. Phys. Chem.* **222,** 65–69.
60. Bellows, J. G. & Shoch, D. E. (1950) *Am. J. Ophthalmol.* **33,** 1555–1564.
61. Kinsey, V. E. & Merriam, F. C. (1950) *Arch. Ophthalmol.* **44,** 370–380.
62. Nishigori, H., Hayashi, R., Lee, J. W., Maruyama, K. & Iwatsuru, M. (1985) *Exp. Eye Res.* **40,** 445–451.
63. Wells, W. W., Xu, D. P., Yang, Y. & Rocque, P. A. (1990) *J. Biol. Chem.* **265,** 15361–15364.
64. Dalton, D. A., Russell, S. A., Hanus, F. J., Pascoe, G. A. & Evans, H. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3811–3815.
65. Meister, A. (1989) in *Glutathione Chemical, Biochemical, and Medical Aspects*, eds. Dolphin, D., Poulson, R. & Avramovic, O. (Wiley, New York), Part A, pp. 367–474.
66. Meister, A. & Larsson, A. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), 6th Ed., pp. 855–868.
67. Mårtensson, J. & Bolin, T. (1986) *Am. J. Gastroenterol.* **81,** 1179–1184.
68. Mårtensson, J., Larsson, J. & Nordström, H. (1987) *Eur. J. Clin. Invest.* **17,** 130–135.
69. Mårtensson, J., Denneberg, T., Lindell, Å. & Textorius, O. (1990) *Kidney Int.* **37,** 143–149.
70. Campbell, G. D., Jr., Steinberg, M. H. & Bower, J. D. (1975) *Ann. Intern. Med.* **82,** 810.
71. Han, J. & Griffith, O. W. (1990) *FASEB J.* **4,** A2049 (abstr.).
72. Griffith, O. W., Han, J. & Mårtensson, J. (1991) *FASEB J.* **5,** in press.