Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9360–9364, October 1991
Biochemistry

Inhibition of glutathione synthesis in the newborn rat: A model for endogenously produced oxidative stress

(ascorbate/mitochondria/antioxidants/buthionine sulfoximine/glutathione ester)

JOHANNES MÅRTENSSON*, AJEY JAIN†, EINAR STOLE*, WILLIAM FRAYER†, PETER A. M. AULD†, AND ALTON MEISTER*

Departments of *Biochemistry and †Pediatrics, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

Contributed by Alton Meister, July 25, 1991

ABSTRACT A model for oxidative stress is described in which glutathione (GSH) synthesis is selectively blocked in newborn rats by administration of L-buthionine-(*S*,*R*)-sulfoximine (BSO). In this model, the normal endogenous physiological formation of reactive oxygen species is largely unopposed, and therefore oxidative tissue damage occurs; because GSH is used for reduction of dehydroascorbate, tissue ascorbate levels decrease. In lung there are decreased numbers of lamellar bodies and decrease of intraalveolar surfactant. Proximal renal tubular, hepatic, and brain damage also occur. A diastereoisomer of BSO that does not inhibit GSH synthesis, L-buthionine-*R*-sulfoximine, does not produce toxicity; this control experiment renders it unlikely that the observed effects of BSO are produced by the sulfoximine moiety itself. There is correlation between the decrease of mitochondrial GSH levels and mitochondrial and cell damage. Oxidative stress as evaluated by mitochondrial damage and mortality can be prevented by treatment with GSH esters or ascorbate. There is apparent linkage between the antioxidant actions of GSH and ascorbate. This model, which may readily be applied to evaluation of the efficacy of other compounds in preventing oxidative stress, offers an approach to study of other effects of GSH deficiency (e.g., on lipid metabolism, hematopoiesis), and closely resembles oxidative stress that occurs in certain human newborns and in other clinical states.

Glutathione (GSH), the ubiquitous peptide thiol that provides cells with their reducing environment, is a key component of the antioxidant system (which includes ascorbate, α-tocopherol, and other compounds) that defends cells against the toxic effects of oxygen (1–3). GSH is synthesized within cells; its export by many cells is a step in a recycling pathway that seems to protect cell membrane components against oxidative damage (4–6). The functions of GSH may be probed by examining the effects of decreasing cellular GSH. Cellular GSH may be decreased by administering compounds that react with GSH to form conjugates or that oxidize GSH to GSSG; however, these approaches are limited by lack of specificity of the reagents available and, because the effects obtained are transient, are associated with major perturbations of metabolism, or both. Inhibition of GSH synthesis by inhibition of γ-glutamylcysteine synthetase [rather than of GSH synthetase, whose blockage leads to metabolic acidosis (7, 8)] is the preferred approach to a sustained decrease in cellular GSH (9). L-Buthionine-(*S*,*R*)-sulfoximine (BSO) and related sulfoximines are highly selective inactivators of γ-glutamylcysteine synthetase, and their administration to animals turns off cellular GSH synthesis effectively (10–13). BSO does not react with GSH, and there is no evidence that the sulfoximine moiety itself exerts toxicity, a conclusion supported by studies reported here on a diastereoisomer, L-buthionine-(*R*)-sulfoximine, that does not inhibit GSH synthesis. The effects observed after treatment with BSO, which may be ascribed to GSH deficiency, are produced by the reactive species that are formed in normal metabolism. Thus, the BSO-model of oxidative stress differs significantly from those in which oxidative stress is produced by radiation or by giving compounds that produce oxidation.

In the initial studies in which adult rats and mice were treated with BSO, the tissue levels of GSH decreased substantially without apparent morbidity (14). Later studies showed that treatment of adult mice with BSO for 7–21 days led to significant damage to skeletal muscle (15), lung type 2 cells (16), and epithelial cells of the jejunum and colon (17). Cellular damage was associated with major destruction of mitochondria [which do not synthesize GSH but transport it from the cytosol (18, 19)]. No damage occurred when GSH monoester, a cellular GSH delivery agent (20–23), was given together with BSO. Mitochondrial degeneration may be ascribed to accumulation of hydrogen peroxide and consequent oxidative damage; a significant fraction of the oxygen used by mitochondria, which do not contain catalase, is normally converted to hydrogen peroxide (24–27). The finding that treatment of newborn mice with BSO produced cataracts (28) led to studies on newborn mice and rats in which it was found that cataracts, which are formed after giving a small dose of BSO, may be prevented by giving GSH monoester (29). BSO is poorly transported into the brains of adult animals and thus has only a small effect on the brain GSH level (30, 31). However, treatment of newborn rats [which have an undeveloped blood brain barrier (30)] with BSO led to brain dysfunction associated with striking enlargement and degeneration of cerebral cortex mitochondria—effects that were prevented by giving GSH monoester (31). GSH deficiency in newborn rats decreases tissue ascorbate levels, and administration of ascorbate, which spares GSH, decreases mortality in GSH-deficient newborn rats (32) and prevents tissue damage. Thus, oxidative damage associated with GSH deficiency can be prevented by either GSH or ascorbate.

In the present work we have further examined the effects of GSH deficiency in newborn rats because this model appears useful for characterization of oxidative stress in several tissues and may have relevance to such stress in the newborn human and in other clinical states.

EXPERIMENTAL SECTION

Materials. Sprague–Dawley rats (female, timed pregnant; also both sexes, newborn to 180 days old) were obtained from Taconic Farms and given laboratory chow and water *ad libitum*. Newborns were breast fed for 18 days. BSO (10–13)

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.

Abbreviations: BSO, L-buthionine-(*S*,*R*)-sulfoximine; GSH, glutathione; GSSG, GSH disulfide; GSSR, GSH–protein mixed disulfide(s).

was recrystallized from 80% (vol/vol) ethanol in 90% yield and fractionally crystallized from water to obtain the less soluble diastereoisomer, L-buthionine-(*R*)-sulfoximine; this product contained <1% of the L-(*S*) isomer (33–35). GSH monoethyl ester (20–23) was obtained as the semi-hydrosulfate salt (23) or free base (36).

Methods. The rats were anesthetized, and the tissue and plasma samples were obtained as described (15, 16, 29, 37). For isolation of lymphocytes and granulocytes, the described (16) method was modified by adding 1 vol of 6% dextran in saline to the whole blood to facilitate removal of reticulocytes by sedimentation. Mitochondria were isolated as described (15–17, 29, 38, 39). Total GSH was determined by the GSSG (GSH disulfide) reductase–DTNB (5,5′-dithiobis(2-nitrobenzoic acid) recycling procedure (37). GSSG was determined by the vinylpyridine method (40). GSH–protein mixed disulfides (GSSR) were determined after reduction with dithiothreitol (37, 41). γ-Glutamylcysteine synthetase was determined as described (15, 16) except that fractionation with ammonium sulfate (31.3 g/100 ml) (42, 43) was done to remove interfering proteins. γ-Glutamyl transpeptidase was determined as described (44). Electron and light microscopy were performed as described (15, 16, 32). Erythrocyte contamination was assessed (16, 45) and found to account for <0.5% of the content of GSH or γ-glutamylcysteine synthetase (16). Protein was determined (46, 47) by using bovine serum albumin as the standard.

RESULTS

Effects of BSO-Induced GSH Deficiency on Newborn Rats. Treatment of newborn rats with BSO (two daily doses of 3 mmol/kg of body weight) led to 83% mortality after 7 days (Table 1). It is important to note that no mortality occurred after administration of the same dose of the L-(*R*) isomer of BSO (Table 1, experiment 4). Mortality was not affected by coadministration of GSH (experiment 5) but was significantly decreased when GSH ester was given together with BSO (experiment 6). As noted previously (29, 31), GSH ester itself is somewhat toxic to newborn rats. Mortality was substantially decreased by coadministration of ascorbate (2 mmol/kg per day) (experiment 8). Higher (5 mmol/kg) doses of ascorbate (or of GSH) are fatal to newborn rats. Doses of 6–8 mmol/kg of BSO or of ascorbate, GSH, and GSH esters can be given to 16-day-old and older rats without mortality.

Administration of BSO led to substantial decline of the mitochondrial GSH levels of lung, kidney, brain, and liver

Table 1. Mortality associated with BSO-induced GSH deficiency in newborn rats

Exp.	Treatment (dose, mmol/kg per day)	Mortality	
		No. died/total	%
1	Saline	0/12	0
2	BSO, L-(*S*,*R*) (6)	30/36	83
3	BSO, L-(*S*,*R*) (8)	20/20	100
4	BSO, L-(*R*) (6)	0/12	0
5	BSO, L-(*S*,*R*) (6) + GSH (5) + ethanol (5)	10/14	71*
6	BSO, L-(*S*,*R*) (6) + GSH ester (5)	7/22	32
7	GSH ester (5)	6/20	30
8	BSO, L-(*S*,*R*) (6) + ascorbate (2)	5/45	11

BSO isomer was injected i.p. into newborn rats (age, 30–36 h) in isosmolar solution at 9 a.m. and 9 p.m. in two equal doses (total daily dose is indicated in parentheses). GSH monoethyl ester was given in two daily doses of 2.5 mmol/kg at 11 a.m. and 11 p.m.; equivalent amounts of GSH, ethanol, and Na_2SO_4 were given in experiment 5. Equal volumes were injected by including saline in injections. Experiment 8 is from ref. 32; ascorbate (1 mmol/kg) was given at 11 a.m. and 11 p.m.
*Not significantly different statistically from experiment 2.

Table 2. Mitochondrial GSH levels after treatment with BSO, L-(*S*,*R*) or L-(*R*) isomer

Tissue	GSH, % of control	
	L-(*S*,*R*)	L-(*R*)
Lung	3.5 ± 0.8	80 ± 1.6
Kidney	2.4 ± 0.4	50 ± 2.4
Brain	9.9 ± 0.5	59 ± 2.2
Liver	5.6 ± 0.4	82 ± 2.2
Heart	40 ± 4.1	89 ± 4.8

Newborn rats (age, 30–36 h) were injected i.p. with saline (controls) or with the L-(*S*,*R*) or L-(*R*) [containing <1% of L-(*S*)] isomer of BSO in doses of 3 mmol/kg twice daily (9 a.m. and 9 p.m.) for 4 days; analyses were done 2 h after the last injection. Brain tissue was cerebral cortex. Control values were 5–9 nmol/mg of protein. Values are given as the percent of control ± SD (n = 3–5).

(Table 2). In contrast, administration of the L-(*R*) isomer of BSO produced only a small decline in mitochondrial GSH levels, an effect consistent with a slight (<1%) contamination of the L-(*R*)-isomer preparation by the L-(*S*) isomer. [Highly purified preparations of the L-(*R*) isomer do not inactivate γ-glutamylcysteine synthetase]. Treatment with BSO [L-(*S*,*R*)] did not affect heart mitochondrial GSH levels to the marked extent found in other tissues.

Administration of BSO to newborn rats led to substantial decline of total tissue GSH levels (Table 3). The decreases were generally greater in newborn than in 16-day-old rats. The level of GSH in lens was most markedly affected.

Electron microscopy revealed decreased numbers of mitochondria in brain, liver, lung, and kidney. In all tissues, except the heart, there was substantial evidence of mitochondrial swelling and degeneration. The lung type 2 cells had decreased numbers of lamellar bodies with decreased intraalveolar tubular myelin and capillary endothelial damage (Fig. 1 *a* and *b*). The giant lamellar bodies may reflect a defect in maturation. There were decreased numbers of lysosomes. The kidney showed mitochondrial damage (32), degeneration of microvilli with nuclear chromatin condensation, and margination associated with necrosis of the proximal tubular cells (Fig. 1*d*). Extramedullary hematopoesis, normally found in the liver of 5-day-old rats, was depressed, and this was especially evident in the erythroblast and megakaryocyte populations (32). None of these effects was found after treatment of the rats with BSO plus GSH ester or ascorbate.

GSH Status of Newborn and Older Rats. The tissue levels of GSH, γ-glutamylcysteine synthetase, and γ-glutamyl trans-

Table 3. Effect of BSO on GSH levels of tissues of newborn and 16-day-old rats

Tissue	GSH, μmol/g of tissue (% of control)	
	Newborn	16 days old
Liver	0.28 ± 0.02 (7.4%)	2.60 ± 0.11 (36%)
Kidney	0.34 ± 0.02 (11%)	0.62 ± 0.03 (27%)
Lung	0.50 ± 0.05 (31%)	0.80 ± 0.04 (39%)
Plasma*	2.20 ± 0.10 (7.6%)	4.40 ± 0.20 (24%)
Heart	0.10 ± 0.01 (4.4%)	0.38 ± 0.02 (19%)
Skeletal muscle	0.12 ± 0.01 (8.7%)	0.09 ± 0.01 (5.7%)
Granulocytes†	1.09 ± 0.11 (65%)	1.06 ± 0.05 (78%)
Lymphocytes†	0.32 ± 0.03 (63%)	0.35 ± 0.02 (73%)
Lens	0.11 ± 0.03 (1.4%)	0.78 ± 0.03 (11%)
Brain‡	(11%)	(59%)

BSO was given (2 mmol/kg) i.p. daily at 10 a.m. for 9 days. Analyses (37) were done 2 h after the last BSO injection. Controls were given saline. Data are given as means ± SD (n = 4 or 5).
*Micromolar GSH from right ventricle.
†nmol of GSH per 10^6 cells.
‡Cerebral cortex; data are from ref. 31. The dose of BSO was 4 mmol/kg per day.

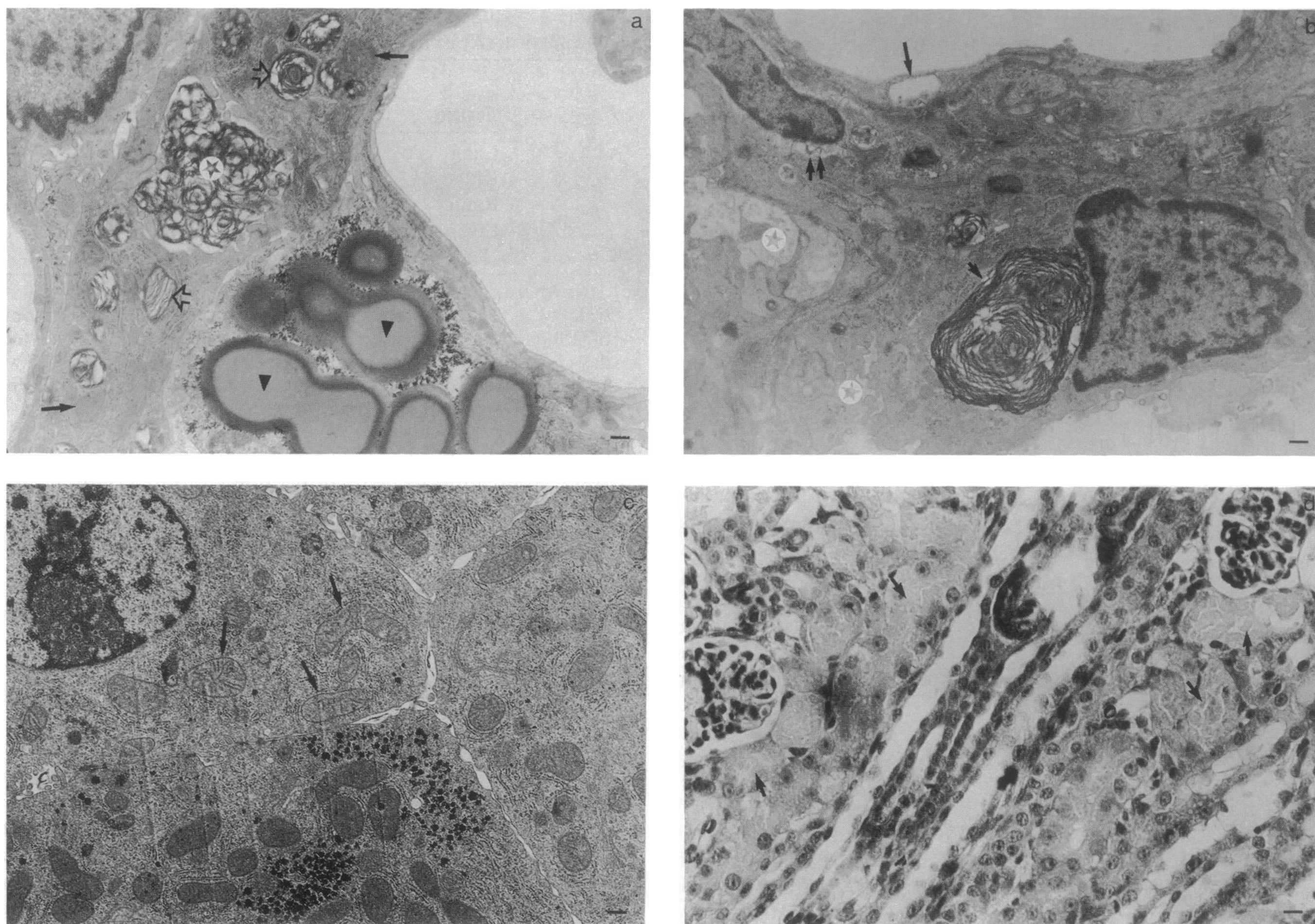

FIG. 1. Electron (a–c) and light (d) microscopy of tissues from 5-day-old rats after treatment with BSO (6 mmol/kg of body weight per day) for 4 days or with saline (control) (a). (a) Saline-treated lung type 2 cells; mitochondria (arrows), lamellar bodies (open arrows), intraalveolar tubular myelin (star), and lysosomes (arrowheads). (×5300; bar = 0.24 μm.) (b) BSO-treated lung type 2 cells; lamellar body giant formation (arrowhead), microvillus blunting and decreased tubular myelin (stars), capillary endothelial swelling with vacuolization (arrow), perinuclear vacuolization (two arrows), and margination of nuclear chromatin. (×5300; bar = 0.24 μm.) (c) BSO-treated liver; focal damage with mitochondrial damage (arrows) and decreased glycogen. (×7400; bar = 0.17 μm.) (d) BSO-treated kidney; proximal tubular degeneration (arrows). (×500; bar = 2.67 μm.)

peptidase in rats of various ages are given in Fig. 2 (see also refs. 48–55). Substantial levels of GSH are present in tissues at all ages. Notably, the level of GSH in the liver increases about 4-fold during development. The level declines in some tissues (e.g., lung, liver, and kidney) shortly after birth; in general, levels then increase to reach adult values.

Separate experiments showed that the GSH levels of the lung decrease by about 30% within the first 2 h of life and then gradually return to adult levels. The mean plasma GSH difference between samples obtained from the right ventricle and the carotid artery was 0.7 μM for 1-day-old rats and 1.8 μM for adult rats (compare ref. 16). These values indicate utilization of plasma GSH of about 6.5 mmol/min per g of lung and 9.0 nmol/min per g of lung in 1-day-old and adult rats, respectively; these values are based on pulmonary plasma flow values of 9.2 ml/min per g of body weight in 1-day-old and 5.0 ml/min per g of body weight in adult rats (56).

Studies on the levels of GSH, GSSG, and mixed disulfides between GSH and proteins (GSSR) in the lung and heart showed that, although the levels of GSSG were similar in 1-day-old and adult rats (about 1% of the total), the GSSR levels (about 20% of the totals) were higher in 1-day-old rats than in adult rats. Thus, GSSR values for 1-day-old heart and lung were 0.54 ± 0.18 and 0.41 ± 0.14 μmol/g, respectively, whereas the corresponding values for adults were 0.18 ± 0.06 and 0.26 ± 0.05 μmol/g. GSSG levels (in plasma) from the right ventricle (0.17 ± 0.11 μM) were lower than those taken from the carotid artery (0.73 ± 0.24 μM) in 1-day-old rats, indicating significant output of GSSG by lung, which was not seen in adults. Plasma GSSR levels were 30–45% of the totals in both newborn and adult rats.

The γ-glutamylcysteine synthetase activity of most tissues of newborns was at or above adult levels except for the kidney, which exhibited about 13% of the adult level at birth. Relatively low γ-glutamyl transpeptidase activity was found in the lung at birth; this activity increased markedly with age. Liver transpeptidase activity was high at birth as previously found (49–52, 55) and decreased rapidly to adult levels. In contrast, the kidney activity is low at birth (49, 50) and increases with age.

The levels of GSH in the liver, plasma, lung, and heart increased in 1-day-old rats after administration of cysteine and to a lesser extent after glutamate and α-ketoglutarate were given; glycine supplementation did not affect GSH levels (Table 4). In similar experiments in which equivalent amounts of GSH were administered, the results were similar to those found after injection of cysteine. That giving either cysteine or GSH increases tissue GSH levels indicates that the availability of cysteine moieties is probably the limiting factor in GSH synthesis in newborn rats as it is in certain adult tissues.

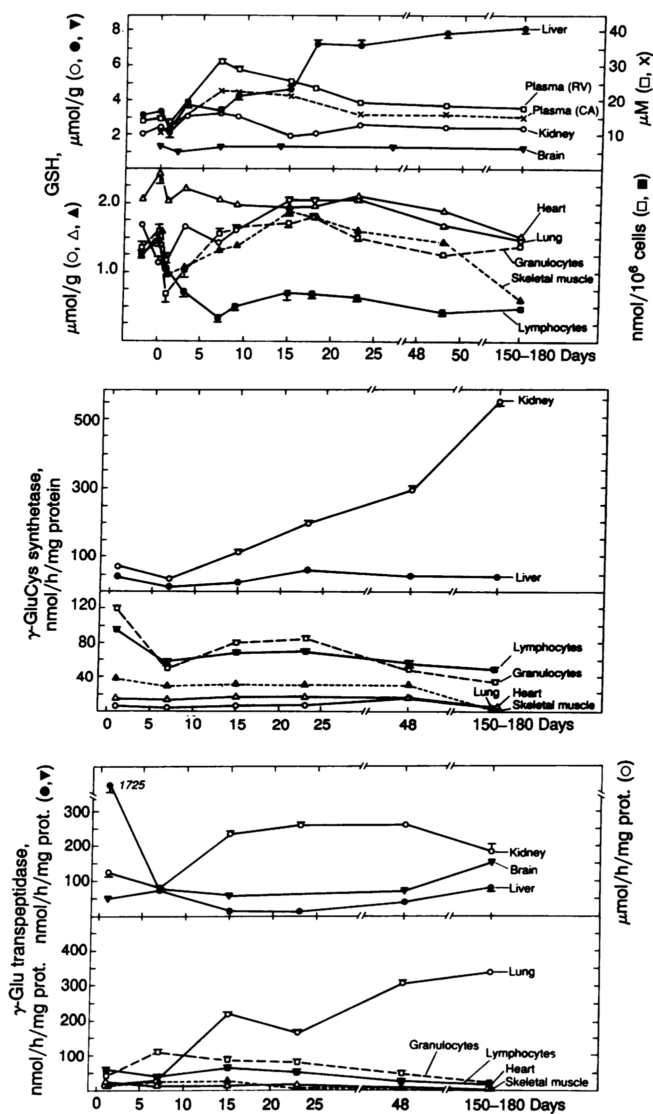

FIG. 2. Levels of GSH (*Top*), γ-glutamylcysteine synthetase (*Middle*), and γ-glutamyl transpeptidase (*Bottom*) in tissues of rats of various ages. Values are means ± SD (n = 3–7).

DISCUSSION

The antioxidant factors that protect the newborn during the rapid transition from uterine to extrauterine life are present at birth (57, 58), and indeed all tissues examined here have appreciable levels of GSH, γ-glutamylcysteine synthetase, and γ-glutamyl transpeptidase. However, there is apparently some "strain" on the system shortly after birth as indicated by decreased levels of GSH in lung and certain other tissues, significant output of pulmonary plasma GSSG, and increased levels of GSSR in lung and heart. Some authors refer to "layers" of antioxidant protection and list them as primary, secondary, and so forth. GSH reacts directly with reactive oxygen intermediates and also supplies antioxidant effects less directly by maintaining in reduced forms various compounds such as ascorbate and α-tocopherol. The major importance of GSH in antioxidant defense is emphasized by the finding of significant tissue damage and mortality after administration of BSO. The finding that the L-(*R*) diastereoisomer of BSO does not produce toxicity is an important control experiment whose results render unlikely the possibility that the effects of BSO are due to another type of toxicity.

Table 4. GSH levels in 1-day-old rats

	GSH, μmol/g of tissue		
Supplement	Liver	Lung	Heart
None	2.80 ± 0.21	1.17 ± 0.23	1.68 ± 0.11
Cysteine	9.05 ± 0.50*	2.20 ± 0.22*	3.20 ± 0.29*
Glutamate	4.22 ± 0.50*	1.25 ± 0.12	2.18 ± 0.20
α-Ketoglutarate	3.61 ± 0.31*	1.05 ± 0.10	1.75 ± 0.15
Cys + Glu + Gly	9.35 ± 0.70*	2.30 ± 0.25*	3.30 ± 0.30*

Tissue samples were taken at 10 a.m. 1 h after i.p. injection of saline or supplement (2 mmol/kg) in isosmolar solution (pH 6.8–7.0). Supplementation with glycine alone had no effect. Studies at 30 min and 2 h after injection showed less effect. Data are means ± SD (n = 4 or 5). Similar data were obtained for plasma (right ventricle). *Significant change ($P < 0.05$) by the Mann–Whitney U test.

Studies on GSH deficiency in adult mice (15–17) and newborn rats (29, 31, 32) indicate that mitochondrial damage is a major consequence of GSH deficiency. There is good correlation between the decrease of GSH levels and occurrence of mitochondrial damage (Fig. 3). GSH-deficient adult mice exhibit severe depletion of GSH in mitochondria in association with mitochondrial and cell damage in lung, jejunum, colon, and skeletal muscle. Damage was not found in liver, heart, kidney, and brain. Cell damage does not occur until the mitochondrial level of GSH is substantially less than 50% of the control, and may be ascribed to accumulation of reactive oxygen compounds and their diffusion into the cytosol, as well as to decreased mitochondrial function with consequent decrease in ATP formed. The factors that determine the variation in mitochondrial GSH deficiency among the various tissues need to be explored. The resistance of the heart and the stomach (17) to GSH depletion is notable and needs further study. Failure of BSO to readily cross the blood–brain barrier undoubtedly protects the brain (31, 59). The relatively high sensitivity of newborn tissues to GSH deficiency as compared with the adult also requires study. Increased retention of BSO by the newborn (32), perhaps due to decreased renal clearance, and also the likelihood that there is little or no synthesis of ascorbate in the newborn rat (see below) need to be considered.

The effects observed on the kidney and lung are notable since these organs are among the least developed at birth, at which time they undergo rapid maturation (60–62). Major developmental changes also occur at this time in liver and brain. GSH deficiency in adult mice leads to lamellar body abnormalities (28) and in newborn rats leads to a decreased number of lamellar bodies and reduced amounts of intraalveolar tubular myelin, which is secreted by the lamellar bodies (Fig. 1 *a* and *b*). This points to a deficiency of surfactant, which might lead to oxidative lung injury (63). Proximal renal tubular cells are highly sensitive to hypoxia, and proximal tubular necrosis (Fig. 1*d*) may partly be a consequence of pulmonary failure (64). Thus, mortality of

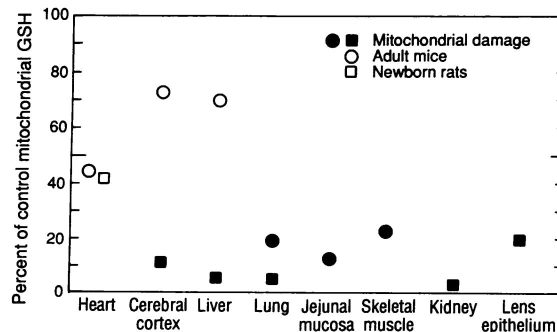

FIG. 3. Mitochondrial damage and GSH levels in tissues of newborn rats and adult mice.

GSH-deficient newborn rats may be ascribed to functional defects in one or more organs. Hyperoxia would be expected to accentuate the type of toxicity observed here; data indicating increased mitochondrial formation of oxygen radicals during hyperoxia have been reported (65).

The present model of oxidative stress, which resembles effects seen in humans deficient in GSH synthesis and GSSG reductase (8), is of interest in relation to other physiological phenomena associated with GSH metabolism [e.g., cholesterol and triglyceride accumulation, decreased hematopoesis (32)].

The effects found here may be ascribed to accumulation of reactive oxygen species normally formed in metabolism, largely by mitochondria. This is consistent with the efficient protection afforded by GSH and ascorbate, which exhibit very similar antioxidant properties. The actions of these compounds are linked because GSH is used for reduction of dehydroascorbate (32, 66). The observed protection of newborn rat lens against cataracts by dehydroascorbate (32) may probably be ascribed to its reduction by GSH in liver (or other organs) and transport of ascorbate via the plasma to the lens. Treatment of adult mice with BSO leads to decrease of ascorbate levels in several tissues except the liver, whose ascorbate level increases for several days (correlated with decrease of GSH levels) followed by a marked decrease (unpublished data). Such induction of ascorbate synthesis in liver does not occur in newborn rats, nor does it occur in guinea pigs (67, 68), which (like humans) do not synthesize ascorbate. It is of interest that guinea pigs are highly sensitive to GSH depletion and, like newborn rats, die soon after receiving several doses of BSO (67). Ascorbate may function physiologically in reactions in which GSH does not efficiently participate and vice versa. It is of interest that these antioxidants can replace each other under certain conditions. The linkage between the antioxidant effects of ascorbate and GSH seems to be of physiological importance; this needs to be further explored.

The authors thank Dr. D. A. Fischman and Mrs. L. Cohen-Gould for assistance in the electron microscopy studies and Drs. H. T. Nguyen and T. Goodwin for kind help with the light microscopy studies. This research was supported in part by a grant to A.M. from the National Institutes of Health (2 R37 DK-12034). J.M. acknowledges stipendary support from the Draco Medical Research Fund.

1. Larsson, A., Orrenius, S., Holmgren, A. & Mannervik, B., eds. (1983) *Functions of Glutathione: Biochemical, Physiological, Toxicological, and Clinical Aspects* (Raven, New York).
2. Meister, A. & Anderson, M. E. (1983) *Annu. Rev. Biochem.* **53,** 711–760.
3. Dolphin, D., Poulson, R. & Avramovic, O., eds. (1989) *Glutathione: Chemical, Biochemical, and Medical Aspects* (Wiley, New York), Parts A and B.
4. Griffith, O. W. & Meister, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 268–272.
5. Anderson, M. E. & Meister, A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 707–711.
6. 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.
7. Wellner, V. P., Sekura, R., Meister, A. & Larsson, A. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2505–2509.
8. 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), pp. 844–868.
9. Meister, A. (1983) *Science* **220,** 471–477.
10. Griffith, O. W., Anderson, M. E. & Meister, A. (1979) *J. Biol. Chem.* **254,** 1205–1210.
11. Griffith, O. W. & Meister, A. (1979) *J. Biol. Chem.* **254,** 7558–7560.
12. Meister, A. (1978) in *Enzyme-Activated Irreversible Inhibitors*, eds. Seiler, N., Jung, M. L. & Koch-Weser, J. (Elsevier–North Holland, Amsterdam), pp. 187–211.
13. Griffith, O. W. (1982) *J. Biol. Chem.* **257,** 13704–13712.
14. Griffith, O. W. & Meister, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6319–6322.
15. Mårtensson, J. & Meister, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 471–475.
16. Mårtensson, J., Jain, A., Frayer, W. & Meister, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5296–5300.
17. Mårtensson, J., Jain, A. & Meister, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1715–1719.
18. Griffith, O. W. & Meister, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4668–4672.
19. Mårtensson, J., Lai, J. C. K. & Meister, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7185–7189.
20. Puri, R. N. & Meister, A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4714–4717.
21. Wellner, V. P., Anderson, M. E., Puri, R. N., Jensen, G. L. & Meister, A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4732–4735.
22. Anderson, M. E., Powrie, F., Puri, R. N. & Meister, A. (1985) *Arch. Biochem. Biophys.* **239,** 538–548.
23. Anderson, M. E. & Meister, A. (1989) *Anal. Biochem.* **183,** 16–20.
24. Loschen, G., Flohé, L. & Chance, B. (1971) *FEBS Lett.* **18,** 261–264.
25. Boveris, A. & Chance, B. (1973) *Biochem. J.* **134,** 707–716.
26. Forman, H. J. & Boveris, A. (1982) in *Free Radicals in Biology*, ed. Pryor, W. A. (Academic, New York), Vol. 5, pp. 65–90.
27. Boveris, A., Oshino, N. & Chance, B. (1972) *Biochem. J.* **128,** 617–630.
28. Calvin, H. I., Medvedovsky, C. & Worgul, B. V. (1986) *Science* **233,** 553–555.
29. Mårtensson, J., Steinherz, R., Jain, A. & Meister, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8727–8731.
30. Slivka, A., Spina, M. B., Calvin, H. I. & Cohen, G. (1988) *J. Neurochem.* **50,** 1391–1393.
31. Jain, A., Mårtensson, J., Stole, E., Auld, P. A. M. & Meister, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1913–1917.
32. Mårtensson, J. & Meister, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4656–4660.
33. Meister, A. (1989) in *Glutathione Centennial: Molecular Properties and Clinical Implications*, eds. Taniguchi, N., *et al.* (Academic, New York), pp. 3–21.
34. Griffith, O. W. (1989) in *Glutathione Centennial: Molecular Properties and Clinical Implications*, eds. Taniguchi, N., *et al.* (Academic, New York), pp. 285–300.
35. Suthanthiran, M., Anderson, M. E., Sharma, V. K. & Meister, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3343–3347.
36. Campbell, E. & Griffith, O. W. (1989) *Anal. Biochem.* **183,** 21–25.
37. Anderson, M. E. (1985) *Methods Enzymol.* **113,** 548–555.
38. Nedergaard, J. & Cannon, B. (1979) *Methods Enzymol.* **55,** 3–28.
39. Lai, J. C. K. & Clark, J. B. (1979) *Methods Enzymol.* **55,** 51–59.
40. Griffith, O. W. (1980) *Anal. Biochem.* **106,** 207–210.
41. Mårtensson, J. (1987) *J. Chromatogr.* **420,** 152–157.
42. Orlowski, M. & Meister, A. (1971) *Methods Enzymol.* **17B,** 495–497.
43. Tsui, E. & Yeung, D. (1979) *Experientia* **35,** 1293–1294.
44. Tate, S. S. & Meister, A. (1985) *Methods Enzymol.* **115,** 400–404.
45. Marklund, S. (1979) *Clin. Chim. Acta* **92,** 229–234.
46. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
47. Redenbaugh, M. G. & Turley, R. B. (1986) *Anal. Biochem.* **153,** 267–271.
48. Tate, S. S., Thompson, G. A. & Meister, A. (1976) in *Glutathione: Metabolism and Function*, Krok Foundation Symposium, eds. Arias, I. M. & Jakoby, W. B. (Raven, New York), pp. 45–55.
49. Tate, S. S. & Meister, A. (1975) *J. Biol. Chem.* **250,** 4619–4624.
50. Meister, A., Tate, S. S. & Ross, L. L. (1976) in *The Enzymes of Biological Membranes*, ed. Martinosi, A. (Plenum, New York), Vol. 3, pp. 315–347.
51. Tateishi, N., Higashi, T., Nakashima, K. & Sakamoto, Y. (1980) *J. Nutr.* **110,** 409–415.
52. Taniguchi, M. & Inoue, M. (1986) *J. Biochem.* **100,** 1457–1463.
53. States, B., Foreman, J. W. & Segal, S. (1987) *Pediatr. Res.* **22,** 605–608.
54. Lambert, G. H. & Thorgiersson, S. S. (1976) *Biochem. Pharmacol.* **25,** 1777–1781.
55. Rollins, D., Larsson, A., Steen, B., Krishnaswamy, K., Hagenfeldt, L., Moldéus, L. & Rane, A. (1981) *J. Pharmacol. Exp. Therapeut.* **217,** 697–700.
56. Foster, H. L., Small, J. D. & Fox, J. G., eds. (1983) *The Mouse in Biomedical Research: Normative Biology, Immunology, and Husbandry* (Academic, New York), Vol. 3.
57. Tanswell, A. K. & Freeman, B. A. (1984) *Pediatr. Res.* **18,** 584–587.
58. Frank, L., Bucher, J. & Roberts, R. J. (1979) *J. Appl. Physiol.* **45,** 699–704.
59. Steinherz, R., Mårtensson, J., Wellner, D. & Meister, A. (1990) *Brain Res.* **518,** 115–119.
60. Guignard, J.-P. (1982) *Pediatr. Clin. North Am.* **29,** 777–790.
61. Gleason, C. A. (1987) *Semin. Perinatol.* **11,** 12–21.
62. Aperia, A. & Herin, P. (1975) *Am. J. Physiol.* **228,** 1319–1325.
63. Hawgood, S. & Clements, J. A. (1990) *Am. Soc. Clin. Invest.* **86,** 1–6.
64. Dauber, I. M., Krauss, A. N., Symchych, P. S. & Auld, P. A. M. (1976) *J. Pediatr.* **88,** 851–855.
65. Freeman, B. A. & Crapo, J. D. (1981) *J. Biol. Chem.* **256,** 10986–10992.
66. Wells, W. W., Xu, D. P., Yang, Y. & Rocque, P. A. (1990) *J. Biol. Chem.* **265,** 15361–15364.
67. Han, J. & Griffith, O. W. (1990) *FASEB J.* **4,** A2049 (abstr.).
68. Griffith, O. W., Han, J. & Mårtensson, J. (1991) *FASEB J.* **5,** A4708 (abstr.).