Glutathione deficiency increases hepatic ascorbic acid synthesis in adult mice

(buthionine sulfoximine/dehydroascorbic acid/kidney/lung)

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ABSTRACT   Glutathione deficiency, induced in adult mice by administering buthionine sulfoximine (an inhibitor of glutathione synthesis), led to a rapid and substantial increase in ascorbate in the liver. This effect was apparent 2–4 hr after giving the inhibitor; subsequently, the level of ascorbate decreased and that of dehydroascorbate increased markedly, supporting the conclusion that glutathione functions physiologically to keep ascorbate in its reduced form. In kidney and lung also, ascorbate levels decreased, and dehydroascorbate increased. Increased synthesis of ascorbate in glutathione-deficient adult mice seems to protect against tissue damage. In contrast, newborn rats, which (like guinea pigs and humans) apparently do not synthesize ascorbate, suffer severe damage to liver and other organs; previous studies showed that administration of ascorbate prevents such tissue damage. The findings support the view that the antioxidant actions of glutathione and ascorbate are closely linked and involve a mechanism in which decrease of the glutathione level, perhaps associated with an oxidative event, stimulates ascorbate synthesis.

Previous studies in this laboratory have shown that inhibition of glutathione synthesis in adult mice and newborn rats produced by administration of buthionine sulfoximine (a transition-state inactivator of γ-glutamylcysteine synthetase) leads to decreased tissue levels of glutathione and of ascorbate, associated with significant tissue damage (1–4). In adult mice there was degeneration of skeletal muscle (5), lung type 2 and endothelial cells (6), and the epithelial cells of jejunum and colon (7); no effects were seen in the liver or kidney. In contrast, newborn rats with glutathione deficiency had focal necrosis and decreased extramedullary hematopoiesis in the liver, renal proximal-tubular degeneration, as well as extensive damage to lung type 2 cells with decreased numbers of lamellar bodies and decreased amounts of intraalveolar tubular myelin (2, 3). Tissue damage produced by glutathione deficiency was closely correlated with mitochondrial damage, a finding consistent with accumulation of hydrogen peroxide. Mitochondria normally produce a substantial amount of hydrogen peroxide (8–11), and when there is marked decrease of mitochondrial glutathione, these organelles undergo oxidative degeneration with consequent tissue damage.

Table 1 summarizes studies on the effects of glutathione deficiency on the adult mouse and newborn rat. It is notable that the marked liver and kidney damage found in glutathione-deficient newborn rats (2, 3) was not found by EM study of these tissues of glutathione-deficient adult mice (6). Furthermore, glutathione deficiency in newborn rats leads to mortality (within several days), but this result was not found in adult mice. These observations suggested that newborn rats, like guinea pigs and humans, cannot synthesize ascorbate (2, 3). On the other hand, adult mice appear more able to deal with

Table 1. Effects of glutathione deficiency produced by administration of buthionine sulfoximine

Effect (ref.)	Animal*	
	Newborn rat	Adult mouse
Early mortality (4–6 days) (2, 3)	+	0
Tissue damage†		
Liver (2, 3, 6)	+	0
Lung (1–3, 6)‡	+	+
Kidney (2, 3, 6)	+	0
Lens epithelium (3, 12, 13)§¶	+	0
Cerebral cortex (2–4)§	+	0
Heart (2, 3, 5)	0	0
Skeletal muscle (1, 5)	—‖	+
Jejunal epithelium (7)	—‖	+‡‡
Colon epithelium (7)	—‖	+‡‡
Gastric epithelium (7)	—‖	0
Pancreas (7)**	—‖	0
Lymphocytes (6)	—‖	+

*0, No effect; +, mortality; mitochondrial and other cellular damage was observed by EM.
†Tissue damage associated with marked (>50%) depletion of mitochondrial glutathione; this damage was prevented by administering ascorbate or glutathione monoesters.
‡Greater damage was found in newborn rat than in adult mouse; this damage was associated with decreased formation of surfactant (1).
§This damage was apparently prevented in adult mouse by the blood–eye and blood–brain barriers.
¶Cataract formation (newborn rat).
‖This damage has not yet been characterized. No skeletal muscle damage was found in newborn rats, which die within 4–6 days.
**No evidence of damage was found by EM in adult mice given buthionine sulfoximine (2.5 mmol/kg i.p. and by gastric tube per day) for 7 days.
‡‡Jejunal and colon epithelia were protected also by orally administered (but not i. p. administered) glutathione (7).

glutathione deficiency because they can synthesize ascorbate. The effects of glutathione deficiency in newborn rats (2, 3) and in adult mice (1) can be prevented by administering high doses of ascorbate, indicating that ascorbate can function as an essential antioxidant in the presence of severe glutathione deficiency and that glutathione and ascorbate have actions in common. This result is supported by the observation that administration of ascorbate to buthionine sulfoximine-treated mice and rats leads to an increase of tissue and mitochondrial levels of glutathione (1, 3); thus, ascorbate "spares" glutathione. In the present studies we have examined the ascorbate levels of several tissues of adult mice during the initial phase of induction of glutathione deficiency.

EXPERIMENTAL SECTION

Materials. Mice (male, 25- to 35-g Swiss–Webster; Taconic Farms) were maintained on Purina chow. L-Buthionine-

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(*S,R*)-sulfoximine was obtained as described (14, 26, 27). Ascorbic acid standards were obtained and characterized as described (3).

Methods. Buthionine sulfoximine was administered i.p. (4 mmol/kg of body weight) twice each day (9 a.m. and 9 p.m.), and buthionine sulfoximine was added (20 mM) to the drinking water.

The tissues were obtained as follows. Mice were decapitated, and the thoracic and peritoneal cavities were opened within 1 min. The tissues were perfused from the left ventricle with 10 ml of cold saline after clamping the venous heart input. Perfusion was completed within 1 min, as the lung turned white, and the liver became light brown. The tissues were excised, quickly blotted, and immersed in liquid nitrogen. The frozen samples were weighed and homogenized in 5 vol of 5% sulfosalicylic acid/3 mM Na_2EDTA. Ascorbate and total ascorbate (ascorbate plus dehydroascorbate) (15, 16) were determined as described (3).

RESULTS

In these studies glutathione deficiency was produced in adult mice by administration of a high dose of buthionine sulfoximine. This inhibitor was given in the drinking water and by twice-daily i.p. injections, essentially as has been described (1–7). Under these conditions, there is a marked decrease of the glutathione levels in several tissues, including skeletal muscle (5), liver (6), and lung (1, 6). The level of mitochondrial glutathione decreases to ≈20% or less of the control level in lung (6) and skeletal muscle (5) but remains at ≈60% of the control level in the liver (6).

In the present work, the levels of ascorbate and of total ascorbate (ascorbate plus dehydroascorbate) were determined in the liver initially and at intervals (from 2 hr to 7 days) after starting the buthionine sulfoximine administration. As shown in Fig. 1, there was a prompt and significant increase in the levels of ascorbate and total ascorbate after 2 and 4 hr. Values for ascorbate and total ascorbate were the same, within experimental error, indicating that the dehydroascorbate level was very low initially and after 2 and 4 hr. After 14 hr and subsequently, the levels of ascorbate declined to about the initial level (or to a somewhat higher level at 7 days). The level of total ascorbate remained at relatively high values, which were substantially greater than those for ascorbate, indicating the presence of appreciable levels of dehydroascorbate. The findings indicate that glutathione deficiency rapidly induces ascorbate synthesis, which is maintained for at least 7 days. No such increase of ascorbate occurs after giving buthionine sulfoximine to newborn rats. After treatment of newborn rats with buthionine sulfoximine, ascorbate and total ascorbate levels decline in liver and other tissues (3).

Studies on the kidney (Fig. 2) showed no significant change of the levels of ascorbate and total ascorbate after 2 hr. Subsequently there was a decrease in the ascorbate level (to 20–45% of initial level), but the level of total ascorbate remained at about control level. In the lung, there was a prompt and progressive decline in ascorbate level (Fig. 3). The level of total ascorbate remained at about the initial value for 14 hr and then declined.

DISCUSSION

These studies show that induction of glutathione deficiency is accompanied by an initial rapid increase in ascorbate level in the liver of adult mice; this effect has been briefly described (2). The magnitude of this increase is substantial and is reflected also in the relatively high levels of total ascorbate observed. The levels of dehydroascorbate, indicated by the difference between the levels of ascorbate and total ascorbate
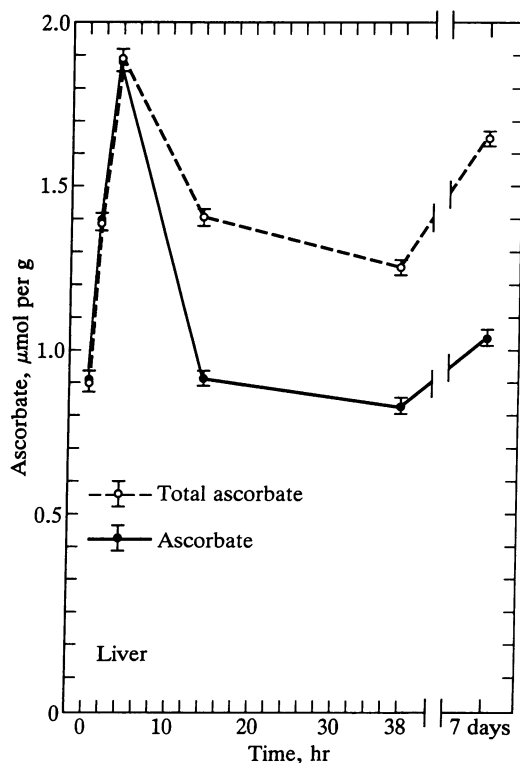

FIG. 1. Levels of ascorbate (●) and total ascorbate (ascorbate plus dehydroascorbate) (○) in livers of adult mice treated with buthionine sulfoximine, as described in text. Initial values were 1.01 ± 0.09 (SD) μmol/g (ascorbate) and 0.99 ± 0.16 μmol/g (total ascorbate); $n = 4$–5.

in Fig. 1, are substantially elevated as compared with initial levels, which are very low. These data, which indicate (*i*) increased ascorbate synthesis and (*ii*) decreased reduction of dehydroascorbate in these glutathione-deficient mice, strongly support the conclusion that an important physiological function of glutathione is to maintain ascorbate in its reduced state (3).

The liver appears to be the chief site of ascorbate synthesis, but in some species (17) ascorbate is synthesized in the
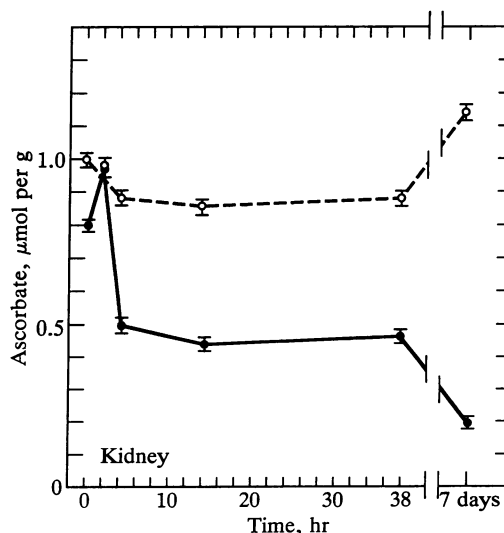

FIG. 2. Levels of ascorbate (●) and total ascorbate (○) in kidneys of adult mice treated with buthionine sulfoximine. Initial values were 0.82 ± 0.07 μmol/g (ascorbate) and 1.04 ± 0.03 μmol/g (total ascorbate).
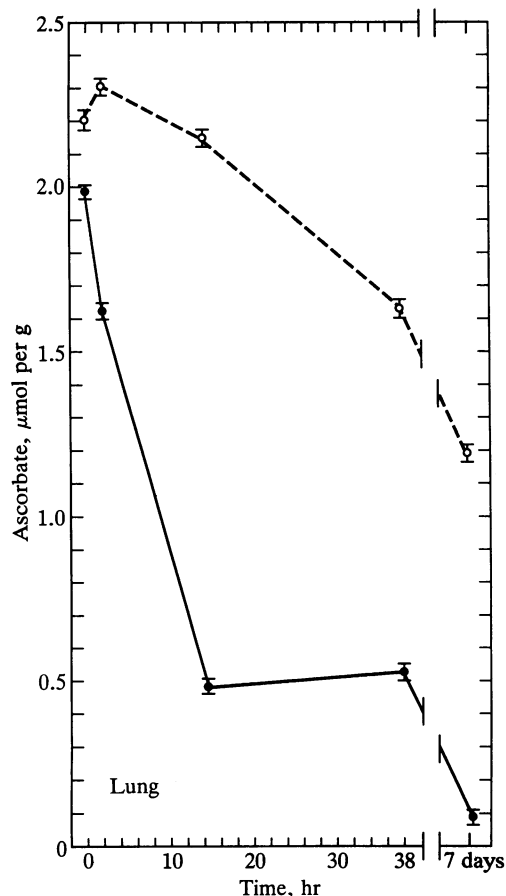
FIG. 3. Levels of ascorbate (●) and total ascorbate (○) in lungs of adult mice treated with buthionine sulfoximine. Initial values were 1.98 ± 0.11 μmol/g (ascorbate) and 2.21 ± 0.15 μmol/g (total ascorbate).

kidney. That the level of total ascorbate is well maintained in the kidney in these experiments (Fig. 2) would be consistent with some renal synthesis of ascorbate or its supply from the liver via the plasma or both. In contrast, the level of total ascorbate in the lung decreases rapidly, suggesting that this organ depends extensively on plasma ascorbate.

Previous studies on newborn rats and adult mice indicate that glutathione and ascorbate function together as an antioxidant couple (2, 3). The present findings on the stimulation of ascorbate synthesis in glutathione deficiency provide further evidence for this conclusion. Additional support comes from recent work in which it was found that the onset of scurvy in ascorbate-deficient guinea pigs was significantly delayed by administration of glutathione monoethyl ester (18). In these studies it was found that ascorbate deficiency in the guinea pig leads to an initial increase of tissue glutathione levels. This result would provide further evidence for the physiological function of the glutathione–ascorbate antioxidant system, which would thus seem to involve symmetrical control mechanisms.

There is much evidence that both glutathione and ascorbate are involved in the metabolism of various drugs. Thus, glutathione is known to form S conjugates with a large variety of chemical agents, some of which can induce synthesis of glutathione and various glutathione-metabolizing enzymes (19–21). There is also considerable literature on the interactions between ascorbate and various drugs, and many studies

have shown stimulation of ascorbate synthesis after drug administration (see, for example, refs. 22–25). The mechanism by which inhibition of glutathione synthesis leads to stimulation of ascorbate synthesis in mouse liver is presently unknown. This mechanism may possibly be similar to one involved in drug-induced ascorbate synthesis. Although this pathway is, of course, not available to humans and guinea pigs, it would be of interest to elucidate the nature of the chemical signal by which a decrease in glutathione triggers ascorbate synthesis. Several intriguing mechanisms can be envisaged to explain how oxidative effects might trigger ascorbate synthesis.

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