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Original Research Communication

GSH Is Required to Recycle Ascorbic Acid in Cultured Liver Cell Lines

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ABSTRACT

Liver is the site of ascorbic acid synthesis in most mammals. As human liver cannot synthesize ascorbate *de novo*, it may differ from liver of other species in the capacity or mechanism for ascorbate recycling from its oxidized forms. Therefore, we compared the ability of cultured liver-derived cells from humans (HepG2 cells) and rats (H4IIE cells) to take up and reduce dehydroascorbic acid (DHA) to ascorbate. Neither cell type contained appreciable amounts of ascorbate in culture, but both rapidly took up and reduced DHA to ascorbate. Intracellular ascorbate accumulated to concentrations of 10–20 m*M* following loading with DHA. The capacity of HepG2 cells to take up and reduce DHA to ascorbate was more than twice that of H4IIE cells. In both cell types, DHA reduction lowered glutathione (GSH) concentrations and was inhibited by prior depletion of GSH with diethyl maleate, buthionine sulfoximine, and phenylarsine oxide. NADPH-dependent DHA reduction due to thioredoxin reductase occurred in overnight-dialyzed extracts of both cell types. These results show that cells derived from rat liver synthesize little ascorbate in culture, that cultured human-derived liver cells have a greater capacity for DHA reduction than do rat-derived liver cells, but that both cell types rely largely on GSH- or NADPH-dependent mechanisms for ascorbate recycling from DHA. Antioxid. Redox Signal. 3, 1089–1097.

INTRODUCTION

ASCORBIC ACID, OR VITAMIN C, is an important antioxidant in plasma and within cells (4, 16). Intracellular recycling of ascorbate derives from both the one- and two-electron-oxidized forms of the vitamin. These are the ascorbate free radical and dehydroascorbic acid (DHA), respectively. Recycling from the ascorbate free radical has been demonstrated in many cell types (20), and this allows ascorbate to be recovered before a second electron removal results in DHA, which is unstable at physiologic pH. Recycling of ascorbate from DHA through glutathione (GSH)-dependent mechanisms has also been described (32), and this can be facilitated by cellular enzymes such as glutaredoxin and protein disulfide isomerase (31, 32). Human erythrocytes appear to use primarily GSH-dependent mechanisms to recycle DHA to ascorbate (14), but some recycling probably occurs through the selenoenzyme thioredoxin reductase in this cell (17). In contrast, ascorbate recycling from DHA in certain nucleated cells, including HL-60 cells (7) and keratinocytes (24), does not require GSH. In rat liver, NADPH-dependent mechanisms may be important, because both thioredoxin reductase (15) and 3α-hydroxysteroid dehydrogenase (3) have been shown to reduce DHA to ascorbate. HepG2 cells, which are derived from a human hepatoma, were shown to take up

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DHA over a period of 30 min and convert it to ascorbate (26). The latter accumulated in the cells to concentrations of as high as 5 m*M* (26). However, the mechanism by which liver or liver-derived cells recycle DHA to ascorbate has not been determined.

In liver, where ascorbate can be synthesized *de novo* in most animals, it is also relevant to consider whether ascorbate recycling might differ depending on the extent to which ascorbate can be synthesized. For example, cells lacking the ability to synthesize ascorbate might have a greater capacity to recycle ascorbate. The present studies were performed to determine the extent to which GSH is required for recycling of DHA to ascorbate in cultured liver-derived cells, and to compare the capacity for recycling in cells derived from rat and human liver, the latter lacking the ability to synthesize ascorbate *de novo*. We found that both cultured rat H4IIE and human HepG2 cells rapidly take up DHA and convert it to ascorbate, which accumulates against a concentration gradient. Both cell types required GSH for optimal reduction of DHA to ascorbate. Although the human-derived cells are unable to synthesize ascorbate *de novo*, they were more effective in DHA uptake and reduction than were cells derived from rat liver.

MATERIALS AND METHODS

Materials

Buthionine sulfoxime (BSO), diethyl maleate, and phenylarsine oxide (PAO) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and the latter two were prepared in dimethyl sulfoxide such that the highest concentration of the latter during cell incubation was 0.8% (vol/vol) or less. Radionuclides were obtained from Amersham Pharmacia Biotech (Piscataway, NJ, U.S.A.). DHA was prepared from ascorbate just before use by bromine oxidation, as previously described (29).

Cell culture

HepG2 cells were kindly provided by Dr. Richard O'Brien (Vanderbilt University) and were grown in Dulbecco's modified Eagle's medium containing 2.5% (vol/vol) newborn calf serum, 2.5% (vol/vol) fetal calf serum, and 5% (vol/vol) Nu Serum IV (Collaborative Research, Inc.). H4IIE cells were a gift from Dr. Daryl Granner (Vanderbilt University) and were cultured in Dulbecco's modified Eagle's medium containing 2.5% (vol/vol) fetal calf serum and 2.5% (vol/vol) newborn calf serum.

Measurement of intracellular water space

Intracellular 3-*O*-[^{3}H]methylglucose was used to estimate the intracellular cytosolic space. Cells in a six-well plate were incubated at 37°C in phosphate-buffered saline (PBS) that contained 1 μCi of 3-*O*-[^{3}H]methylglucose and 0.3 μCi of [^{14}C]mannitol. After 10 min of incubation, an aliquot of the medium was taken for radioactive counting. The cells were then rinsed twice with 2 ml of ice-cold PBS, followed by 1 ml of PBS and removal of the cells from the plate by scraping with a rubber policeman. The cells and buffer were transferred to a scintillation vial that contained 4 ml of liquid scintillation fluid. The vials were counted in a Packard 2000CA liquid scintillation spectrometer under dual label conditions with quench correction. Intracellular cytosolic space was calculated by dividing the fraction of the total tritium counts that were in the cells by the volume of the incubation medium. This was corrected for water trapped outside the cells by subtracting the mannitol space determined in the same manner using the carbon-14 counts. Protein was measured using the D_C protein assay (Bio-Rad, Richmond, CA, U.S.A.). The cytosolic space in HepG2 cells was 1.7 μl/mg of protein (1.05 μl/well of cells, $n = 5$ determinations), and that in H4IIE cells was 2.8 μl/mg of protein (1.13 μl/well of cells, $n = 5$ determinations).

Measurement of DHA uptake and reduction to ascorbate and of GSH

Cells that were cultured in six-well plates were incubated in either culture medium or PBS containing additions as noted at 37°C. PBS consisted of deionized water containing 12.5 m*M* sodium phosphate, 140 m*M* sodium chloride, pH 7.4. Except where noted, incubations were terminated at 30 min by rapid removal of

the medium and one rinse in 2 ml of PBS. The monolayer of cells was treated with 0.5 ml of ice-cold 80% (vol/vol) methanol that contained 1 m*M* EDTA. After several minutes on ice, the cells were removed from the dish by scraping with a rubber policeman. The lysate was removed, and the well was washed again with 0.5 ml of the methanol/EDTA solution. Methanolic solutions were combined, the volume was measured, and cellular debris was pelleted in a microfuge. Aliquots of the supernatant were taken for assay of ascorbate or GSH, as noted. Ascorbate was assayed as previously described (16) using ion-pair high-performance liquid chromatography with electrochemical detection. GSH was measured using the fluorometric assay described by Hissin and Hilf (11).

Measurement of thioredoxin reductase-dependent DHA reduction

The ability of cytosolic thioredoxin reductase to reduce DHA to ascorbate was measured following overnight dialysis of cell extracts as previously described for liver homogenate (15). Cells were scraped from four wells of a plate, suspended in 1.5 ml of PBS, frozen in dry ice-acetone, and allowed to thaw on ice. The lysate was microfuged for 5 min at 3°C at 16,000 *g*, and the supernatant was centrifuged for 1 h at 100,000 *g* in a Beckman T-100 ultracentrifuge. The supernatant was dialyzed overnight against three changes of Tris-EDTA buffer in 3,500 molecular weight cut-off dialysis tubing. The dialysate was used immediately for assay of DHA reduction as previously described (15).

Data analysis and statistical methods

Data are expressed as means ± SD from the indicated number of experiments. Differences between two treatments were analyzed by paired *t* testing, and those between multiple treatments by one-way analysis of variance with post-hoc analysis by Dunnett's test, using the statistical software SigmaStat 2.0 (Jandel Scientific, San Rafael, CA, U.S.A.). Curve-fitting was performed using the graphics analysis program Origin 6.0 (Microcal, Northampton, MA, U.S.A.).

RESULTS

Ascorbate was not detected in HepG2 cells or in the culture medium used for these cells. H4IIE cells had low, but detectable concentrations of intracellular ascorbate (9.8 ± 3 μM, n = 3). However, there was also a small amount of ascorbate found in fresh culture medium that had not been exposed to cells (7 ± 2 μM). Both H4IIE and HepG2 cells rapidly took up DHA from medium and reduced it to ascorbate, as shown in the representative time courses in Fig. 1. Uptake was essentially complete at the 5-min time point for each cell type. HepG2 cells typically accumulated higher intracellular concentrations of ascorbate over the period of incubation than did H4IIE cells.

When the two cell types were incubated for 30 min at 37°C with increasing concentrations of DHA, intracellular ascorbate concentrations rose to 10–20 m*M* (Fig. 2). Ascorbate accumulation was greater in HepG2 cells than in H4IIE cells at every DHA concentration tested. Further, the DHA concentration at which half-maximal DHA uptake and conversion to ascorbate occurred was lower in HepG2 cells (57 ±

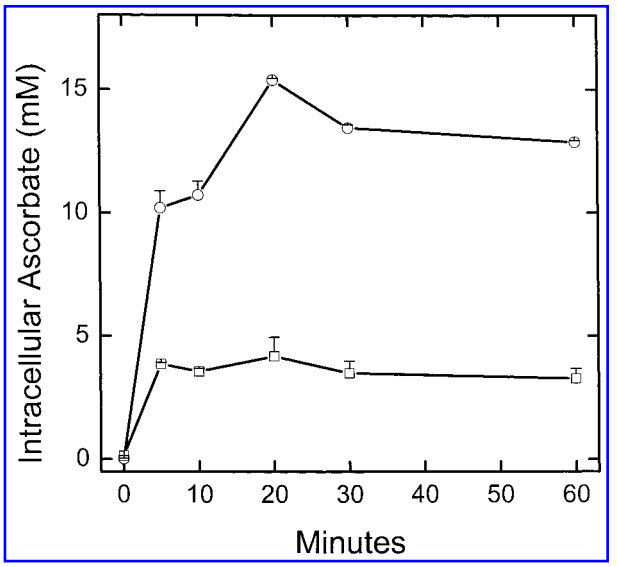

FIG. 1. Time-dependent uptake and reduction of DHA. HepG2 (○) and H4IIE (□) cells were incubated with 0.33 m*M* DHA in culture medium for the times indicated before removal of the medium and assay of ascorbate in the cells. The results shown are means ± SD of duplicate measurements from an experiment representative of three performed.

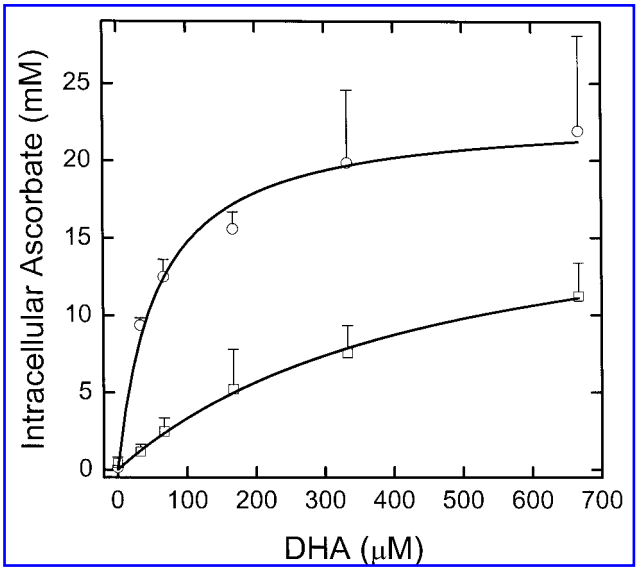

FIG. 2. Concentration dependence of DHA uptake and reduction by HepG2 (○) and H4IIE (□) cells. Cells were treated with the indicated initial concentration of DHA for 30 min in culture medium, followed by assay of intracellular ascorbate. Data are shown as means ± SD from three experiments with each cell type. The DHA concentration of half-maximal uptake and conversion to ascorbate was determined by fitting the data to a rectangular hyperbola and calculating the apparent K_m.

10 μM) than in H4IIE cells (462 ± 70 μM). Given the rapid initial uptake by both cell types (Fig. 1), transport of DHA should not be limiting during this 30-min incubation. Therefore, the difference in ascorbate accumulation probably reflects different capacities for the cells to convert DHA to ascorbate. Despite the remarkable ascorbate accumulation in both cell types, it does appear that the ability of the cells to reduce DHA is limited, because uptake and reduction appeared to be saturable.

The role of GSH in the ability of the cells to reduce DHA to ascorbate was assessed first by incubating cells with a high concentration of DHA (2 mM) in the absence of glucose. If GSH is required for recycling ascorbate, then GSH should be depleted during recycling in the absence of glucose. As shown in Table 1, initial intracellular GSH concentrations were similar in the two cell types. DHA treatment decreased the GSH content of both cell types by ~50%. These results suggest that, for both cell types, either DHA is required for ascorbate recycling, or such recycling induces an oxidant stress that indirectly consumes GSH. A second approach to assess whether GSH is required for ascorbate recycling was to first deplete GSH, then assess the ability of the cells to reduce DHA to ascorbate.

Diethyl maleate was used in the studies shown in Fig. 3 to selectively lower intracellular GSH concentrations. Diethyl maleate is conjugated with GSH by glutathione *S*-transferase, and thereby depletes cellular GSH (19, 22). In these studies, cells were incubated for 10 min with diethyl maleate to deplete GSH, DHA was added, and the ability of the cells to take up and reduce DHA to ascorbate was assessed. If GSH is necessary for DHA reduction, ascorbate concentrations should be less in the presence of increasing diethyl maleate concentrations. As expected, diethyl maleate lowered intracellular GSH in both cell types, although H4IIE cells were much more sensitive than were HepG2 cells to the effects of this agent (Fig. 3A). The ability to reduce DHA to ascorbate was decreased to a similar relative extent in each cell type by pretreatment with diethyl maleate (Fig. 3B). Although both cell types required GSH to optimally reduce DHA to ascorbate, H4IIE cells were better able to sustain DHA reduction in the presence of a more severe GSH depletion.

In another type of experiment, GSH was depleted in both cell lines using treatment in cul-

TABLE 1. EFFECTS OF DHA ON INTRACELLULAR GSH CONCENTRATIONS

Treatment	*HepG2 cells* (n = 4)	*H4IIE cells* (n = 5)
Control	5.2 ± 0.8	7.2 ± 4.9
DHA (2mM)	2.8 ± 0.4*	3.2 ± 4.0*

Cells were rinsed free of media and incubated in PBS that contained DHA and no glucose for 60 min before assay of the cellular GSH concentration (mM). Results are shown as means ± SD.
*$p < 0.02$ compared with control for same cell type.

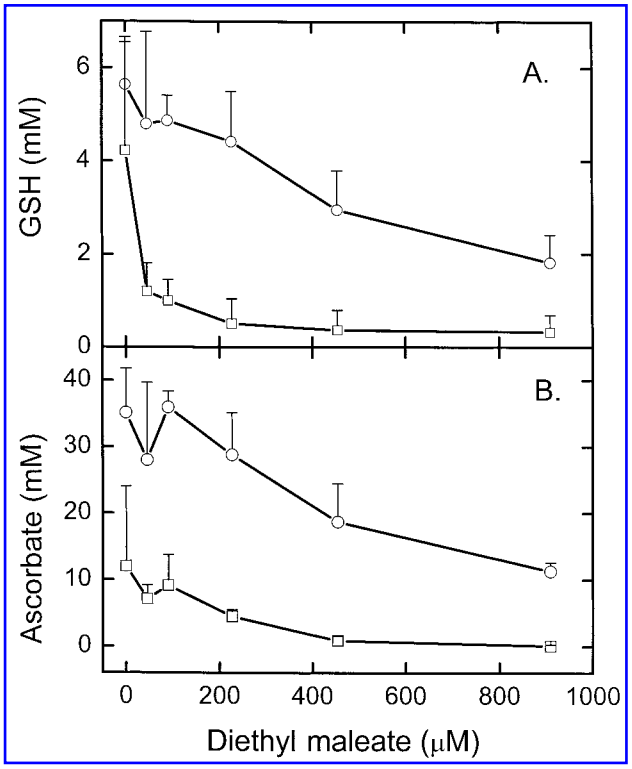

FIG. 3. Effects of diethyl maleate pretreatment of cells on GSH and DHA recycling to ascorbate. HepG2 cells (○) or H4IIE cells (□) were washed twice with 2 ml of PBS to remove medium, and incubated at 37°C in 2 ml of PBS that contained 5 m*M* D-glucose and the indicated concentration of diethyl maleate. After 10 min, DHA was added to an initial concentration of 0.4 m*M* and the incubations were continued for another 30 min followed by removal of the medium and assay of the cell content of GSH (**A**) and ascorbate (**B**). Data are means ± SD from three experiments with each cell type.

ture with BSO, which blocks GSH synthesis (6). As shown in Table 2, following a 24-h treatment with 0.5 m*M* BSO, the GSH content decreased to 25% of control in HepG2 cells, and to 4% of control in H4IIE cells. This was associated with a 35% decrease in the ability of HepG2 cells to reduce DHA to ascorbate, and a 50% decrease for H4IIE cells. The ability to reduce DHA to ascorbate was impaired in proportion to the extent of GSH depletion, again suggesting that GSH is required for DHA reduction.

PAO, which reacts with vicinal sulfhydryl groups, was also tested for its ability to impair cellular reduction of DHA to ascorbate. The design of the experiment shown in Fig. 4 was similar to that for Fig. 3, except that cells were pretreated with PAO for 30 min, than loaded with DHA. In contrast to the results with diethyl maleate, GSH concentrations in HepG2 cells were quite sensitive to PAO, whereas in H4IIE cells, ~40% of cellular GSH remained following DHA loading and treatment with the highest dose of PAO. Again, these results strongly support a GSH- or at least protein-thiol-dependent mechanism for DHA reduction to ascorbate by both cell types. On the other hand, the ability of both cell types to recycle ascorbate decreased essentially to zero at 12.5 μM PAO. The loss of ability to recycle ascorbate when 20–40% of cellular GSH content remained suggests that PAO might affect other thiol-dependent mechanisms of ascorbate recycling.

To determine whether the thioredoxin reductase system might be involved in ascorbate recycling from DHA, the ability of dialyzed cell extracts to carry out NADPH-dependent DHA reduction was assessed, with the results shown in Table 3. Overnight dialysis of the soluble

TABLE 2. INHIBITION OF GSH SYNTHESIS AND DHA REDUCTION IN CULTURED LIVER CELLS

Treatment	*GSH (mM)*	*Ascorbate (mM)*
HepG2 cells	2.4 ± 0.4	60 ± 20
HepG2 cells, BSO-treated	0.6 ± 0.13*	39 ± 16*
H4IIE cells	3.3 ± 1.7	12.9 ± 1.4
H4IIE cells, BSO-treated	0.14 ± 0.04*	6.6 ± 2.8*

Cells in culture were treated where indicated with 0.5 m*M* BSO for 24 h. DHA was added to all samples to a final concentration of 0.4 m*M*, and incubations were continued for another 30 min at 37°C, followed by removal of the media, one rinse with PBS, and assay of GSH and ascorbate contents of the cells. Results are shown as means ± SD from four experiments with each cell type.

*$p < 0.05$ compared with control for same cell type.

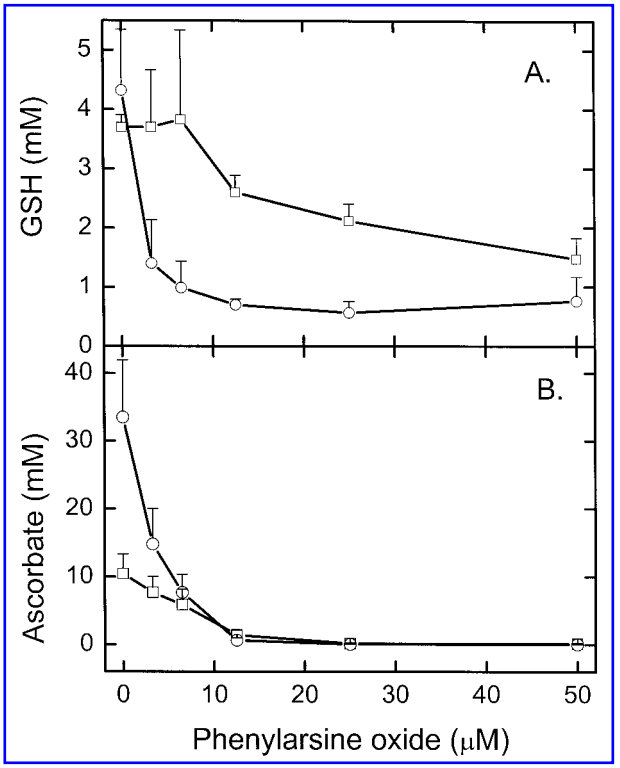

FIG. 4. Effects of PAO pretreatment of cells on GSH and DHA recycling to ascorbate. Treatments and conditions were identical to those described in the legend to Fig. 3, except that the preincubation time with PAO was 30 min. Responses of HepG2 cells are noted by the circles, those of H4IIE cells by the squares. (**A**) GSH results. (**B**) Ascorbate results. Data are shown as means ± SD from three experiments with each cell type.

fractions of cell lysates removed low molecular weight co-factors such as GSH, NADH, and NADPH that might interfere with the assay of thioredoxin reductase. To increase specificity for detecting reduction by thioredoxin reductase, its inhibition by aurothioglucose and stimulation by selenocystine were studied. Aurothioglucose has been shown to selectively inhibit thioredoxin reductase over glutathione peroxidase at a 10 μM concentration (10). Selenocystine enhances the redox capacity of thioredoxin reductase (1). As shown in Table 3, both cell types reduced DHA to ascorbate in a manner consistent with the activity of thioredoxin reductase. Basal and gold-sensitive DHA reduction by the thioredoxin system (thioredoxin reductase plus thioredoxin) was about twice as high in extracts from H4IIE cells as in extracts from HepG2 cells (Table 3). Selenocystine stimulated activity in extracts from both cell types to a similar extent. These results show that cytosolic extracts from both cell types can reduce DHA to ascorbate in a thioredoxin reductase-dependent manner. They also show that the enhanced ability of HepG2 cells to take up DHA and recycle it to ascorbate is not due to thioredoxin reductase.

DISCUSSION

As expected (26), cells derived from a human hepatoma have no endogenous ascorbate when cultured in ascorbate-free medium (Figs. 1 and 2). Surprisingly, rat-liver-derived H4IIE cells have only low micromolar ascorbate concentrations, similar to those measured in the fresh culture media used for these cells. As the concentration of ascorbate in rat liver (15) and isolated hepatocytes (8) is 1 mM or more, our failure to find higher concentrations in H4IIE cells suggests that ascorbate synthesis is minimal in these cells. This could indicate that the ascorbate synthetic mechanism is inactive, that the cells are under oxidant stress, or that the higher levels of ascorbate in hepatocytes prepared from rat liver derive mostly from the diet.

Whereas ascorbate is not taken up by isolated rat hepatocytes during a 60-min incubation at 37°C (2), DHA is rapidly taken up by both hepatocytes (2) and cultured tumor-derived liver cells (Fig. 1) (26). As in other cells, initial DHA uptake likely reflects its transport by facilitated diffusion on the GLUT-type glucose transporters (27). Our findings that DHA uptake and reduction are complete within 5 min (Fig. 1) and that uptake saturates at initial extracellu-

TABLE 3. ROLE FOR THIOREDOXIN REDUCTASE IN DHA RECYCLING IN CELL LYSATES

Treatment	*HepG2 cells*	*H4IIE cells*
Control	31 ± 9	55 ± 20
Selenocystine (100 μM)	135 ± 40*	171 ± 80*
Aurothioglucose (10 μM)	11 ± 11*	15 ± 11*
Gold-sensitive activity†	20 ± 13*	40 ± 16*‡

Values are nmol · (10 min)$^{-1}$ · (mg of dialysate protein)$^{-1}$. Results are shown as means ± SD from five experiments with each cell type.

*$p < 0.05$ compared with control for same cell type.

†Calculated as (control − aurothioglucose) for each cell type.

‡$p < 0.05$ compared with HepG2 cells.

lar DHA concentrations of 57 μM for HepG2 cells and 462 μM for H4IIE cells (Fig. 2) suggest that the reduction step is rate-limiting for overall uptake. As the GLUT-type transporters show apparent K_m values for DHA transport of 1 mM (23), the lower half-maximal values observed in this study imply limitation at a step beyond transport. Further, human erythrocytes have very rapid uptake of DHA on the GLUT1 glucose transporter, and the uptake of DHA does not limit its reduction (14). The concentration of half-maximal DHA uptake and reduction is 400 μM in erythrocytes (18), similar to that observed for H4IIE cells, but higher than that observed for HepG2 cells. This apparent increased affinity for DHA compared with the transport step strongly suggests that reduction of DHA to ascorbate limits its overall uptake.

HepG2 cells accumulate ascorbate to intracellular concentrations that are more than twice as high as those generated in H4IIE cells (Figs. 1 and 2). This, and the observation that DHA uptake and reduction saturate at lower initial extracellular DHA concentrations in HepG2 cells than in H4IIE cells (Fig. 2), suggest that the human-derived cells have a greater capacity for DHA reduction to ascorbate than do the rat-derived cells.

Reduction of DHA to ascorbate is substantially GSH-dependent in both cell types. This conclusion derives from the findings that intracellular GSH concentrations decrease during DHA loading (Table 1), and that the ability of the cells to recycle DHA to ascorbate is impaired in proportion to the GSH content of the cells (Fig. 3). The validity of the latter depends on the specificity of diethyl maleate for GSH. This agent lowers GSH in a conjugation reaction mediated by glutathione *S*-transferase (19). Diethyl maleate has been previously shown to have specificity for GSH over ATP in hepatocytes (22). Additional support for a role of GSH in DHA reduction is the finding that inhibition of GSH synthesis using BSO in culture also decreases the ability of both cell lines to reduce DHA to ascorbate (Table 2). The results from the PAO experiment (Fig. 4) suggest that at least part of the GSH-dependent ascorbate recycling is mediated by an enzyme with sensitive vicinal thiol groups. The findings with these three agents that affect GSH contents by different mechanisms support the contention that GSH, either directly (32) or as a co-substrate for GSH-dependent enzymes (30), is involved in DHA recycling in hepatoma cells from both humans and rats.

The GSH dependence of liver-derived cultured cells and in human erythrocytes contrasts with the lack of such dependence in cultured HL-60 cells (7) and in skin keratinocytes (24). These differences may simply reflect high intracellular concentrations of GSH and a robust capacity for GSH recycling in erythrocytes and liver. They also point out the diversity of cellular mechanisms for ascorbate recycling from DHA, which is underscored by our demonstration of NADPH-dependent DHA recycling in liver-derived cells. Dialyzed extracts from both HepG2 and H4IIE cells reduce DHA to ascorbate (Table 3). As this reduction is NADPH-dependent, inhibited by aurothioglucose, and stimulated by selenocystine, it reflects the activity of thioredoxin reductase. We have previously shown similar results in dialyzed extracts from rat liver, and that selenium deficiency decreases liver ascorbate concentrations by 20–30% (15). Although 70–80% of NADPH-dependent DHA reduction in cytosolic extracts is sensitive to inhibition by aurothioglucose (Table 3), the extent to which thioredoxin reductase mediates DHA reduction in the intact cell cannot be determined from our results. As cultured cells, and HepG2 cells in particular, have been shown to be deficient in selenium (9), it will be necessary to supplement culture media with selenium to determine the extent to which the cultured liver-derived cells use the thioredoxin reductase system to recycle DHA to ascorbate. Nevertheless, the sensitivity of DHA reduction to GSH depletion in cultured liver cell lines suggests that their capacity to reduce DHA is largely GSH-dependent. Further, we previously measured rates of direct reduction of DHA by 2 mM GSH under the conditions used to assay thioredoxin reductase in dialysates (15). Those rates were four- to seven-fold greater than observed for aurothioglucose-sensitive DHA reduction by thioredoxin reductase in the present studies.

The physiologic relevance of DHA uptake and reduction by liver cells is yet to be demonstrated. Although ascorbate uptake by he-

patocytes is much slower than that of DHA (2), hepatocytes do contain mRNA for the newly described ascorbate transport protein (SVCT1) (25). The presence of this sodium- and energy-dependent transporter could account for the accumulation of ascorbate against a concentration gradient that has been observed in cultured hepatocytes (2, 5) and in liver (15). Measured DHA concentrations in human plasma are generally much lower than those of ascorbate (12, 13). On the other hand, hepatocytes or other cell types in liver subjected to acute oxidant stress will release DHA, which could then be rapidly taken up and efficiently reduced to ascorbate by hepatocytes. Such a mechanism is supported by the observations of Cornu *et al.* (2) that the redox cycling agent diquat, when added to hepatocytes in the presence of ascorbate, accelerated ascorbate accumulation within the cells.

In addition to recycling ascorbate from DHA, recent evidence by Upston *et al.* (26) indicates that ascorbate is also released by HepG2 cells in a time- and concentration-dependent manner. Ascorbate release was also documented from perfused rat liver in that study. Inhibition of ascorbate release by phloretin, 4,4′-diisothiocyanostilbene-2,2′-disulfonate, and isoascorbate suggests involvement of a transporter protein, rather than co-release during secretory events (21, 28). DHA uptake and recycling to ascorbate may be important not only for hepatocytes, but also as a mechanism to maintain plasma concentrations of the vitamin (26). Our results document the efficiency of this recycling in cultured liver-derived hepatocytes, and suggest that human cells have a greater capacity for such recycling than animal cells presumably capable of *de novo* ascorbate synthesis.

ABBREVIATIONS

BSO, buthionine sulfoximine; DHA, dehydroascorbic acid; GSH, glutathione; PAO, phenylarsine oxide; PBS, phosphate-buffered saline.

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