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Frank Giblin
Oakland University
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# Glutathione: A Vital Lens Antioxidant

FRANK J. GIBLIN

*Eye Research Institute, Oakland University, Rochester, Michigan*

## ABSTRACT

The reducing compound glutathione (GSH) exists in an unusually high concentration in the lens where it functions as an essential antioxidant vital for maintenance of the tissue's transparency. In conjunction with an active glutathione redox cycle located in the lens epithelium and superficial cortex, GSH detoxifies potentially damaging oxidants such as $H_2O_2$ and dehydroascorbic acid. Recent studies have indicated an important hydroxyl radical-scavenging function for GSH in lens epithelial cells, independent of the cells' ability to detoxify $H_2O_2$. Depletion of GSH or inhibition of the redox cycle allows low levels of oxidant to damage lens epithelial targets such as Na/K-ATPase, certain cytoskeletal proteins and proteins associated with normal membrane permeability. The level of GSH in the nucleus of the lens is relatively low, particularly in the aging lens, and exactly how the compound travels from the epithelium to the central region of the organ is not known. Recently, a cortical/nuclear barrier to GSH migration in older human lenses was demonstrated by Sweeney et al. The relatively low ratio of GSH to protein -SH in the nucleus of the lens, combined with low activity of the glutathione redox cycle in this region, makes the nucleus especially vulnerable to oxidative stress, as has been demonstrated with use of *in vivo* experimental animal models such as hyperbaric oxygen, UVA light and the glutathione peroxidase knockout mouse. Effects observed in these models, which are currently being utilized to investigate the mechanism of formation of human senile nuclear cataract, include an increase in lens nuclear disulfide, damage to nuclear membranes and an increase in nuclear light scattering. A need exists for development of therapeutic agents to slow age-related loss of antioxidant activity in the nucleus of the human lens to delay the onset of cataract.

## INTRODUCTION

Although the role of reduced glutathione (GSH) in the lens has been investigated for at least sixty years (1), exactly how this important antioxidant functions to maintain lens transparency is still not well understood. In 1966, Reddy with Klethi and Kinsey (2), measured the turnover rate of GSH in the rabbit lens and showed, remarkably, that the compound is completely broken down and resynthesized in the tissue about every 48 hr. Subsequently, Reddy and his coworkers demonstrated that all enzymes of the gamma glutamyl cycle are present in the lens epithelium to continuously degrade and synthesize GSH

(3-5). The fact that three molecules of ATP per molecule of GSH are required to complete this process (6) highlights the essential nature of this compound for the normal lens. Review articles on the role of GSH in the lens have been published previously by Reddy and coworkers (6-9).

## The Function of GSH in the Lens Epithelium and Cortex

The cortex of the lens possesses an unusually high level of GSH, amounting to over 20 mM in the rabbit and guinea pig (7,10,11), and the concentration present in the epithelium may be even higher (12). Glutathione in a healthy lens epithelium exists almost entirely in the reduced form with the presence of nearly undetectable levels of oxidized glutathione (GSSG) (10,12). In addition to GSH, the epithelium also contains an active glutathione redox cycle. Through this pathway, glutathione reductase, NADPH and the hexose monophosphate shunt (HMPS) reduce GSSG efficiently back to GSH as it is formed (13-15). The response of HMPS activity in cultured human lenses challenged with the potentially damaging oxidant $H_2O_2$ is shown in Fig. 1. Activity of the shunt increases nearly linearly up to 0.1 mM peroxide with a 20-fold stimulation over the baseline level. Similarly, when a cultured rabbit lens is exposed for 30 min to a high concentration of another oxidant, t-butyl hydroperoxide, GSH is nearly completely oxidized in the epithelium (Fig. 2); however, after a 2 hr culture of the treated lens in normal medium, GSSG is completely returned to the reduced form. These experiments demonstrate that the lens epithelium is capable of maintaining high levels of GSH in the reduced state. Surprisingly, this ability may be observed even in certain types of human senile cataracts. Brunescent cataracts from India, which were found to be completely devoid of GSH in the cortex and nucleus, showed nearly normal amounts of GSH in the epithelium, with no GSSG present (17). Similarly, mature X-ray cataracts in the rabbit, which exhibited nearly 90% loss of GSH in the whole lens, had less than 50% depletion of the compound in the epithelium (18).

One way to demonstrate the remarkable antioxidant capability of the lens epithelium is to challenge cultured lens epithelial cells with a single pulse of a high level of potentially lethal $H_2O_2$. When this is done with rabbit lens cells under conditions described in the legend of Fig. 3, there is, at time zero of the experiment, 60 times more $H_2O_2$ in the culture medium (2,500 nmol) than there is GSH in the cultured cells (40 nmol). However, surprisingly, when the cells are observed three hours later, no cell death or morphological effects are apparent (19). One reason for this phenomenon is that, in spite of the high ratio of $H_2O_2$ to GSH, the concentration of GSH within the lens cells never drops below about 35% of normal

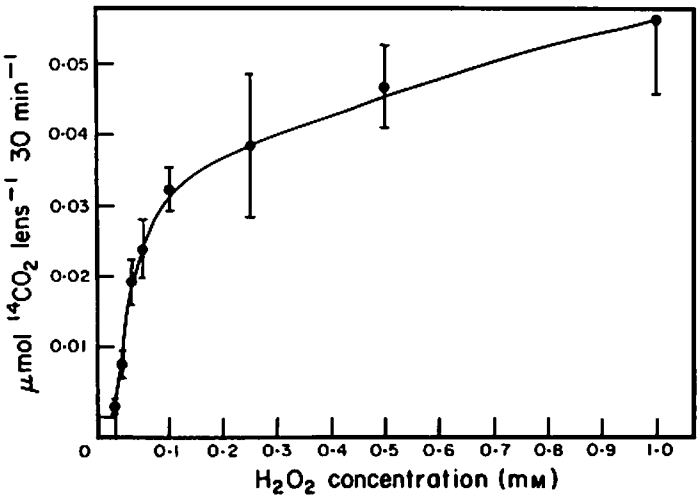


FIGURE 1. The Effect of $H_2O_2$ on the Oxidation of $^{14}C$-1-labeled Glucose in Cultured Human Lenses. Each lens was exposed to various concentrations of $H_2O_2$ from 0 to 1.0 mM for 30 min periods. Results are expressed as the mean ± S.D. for six lenses. Reprinted by permission from Ref. 16.

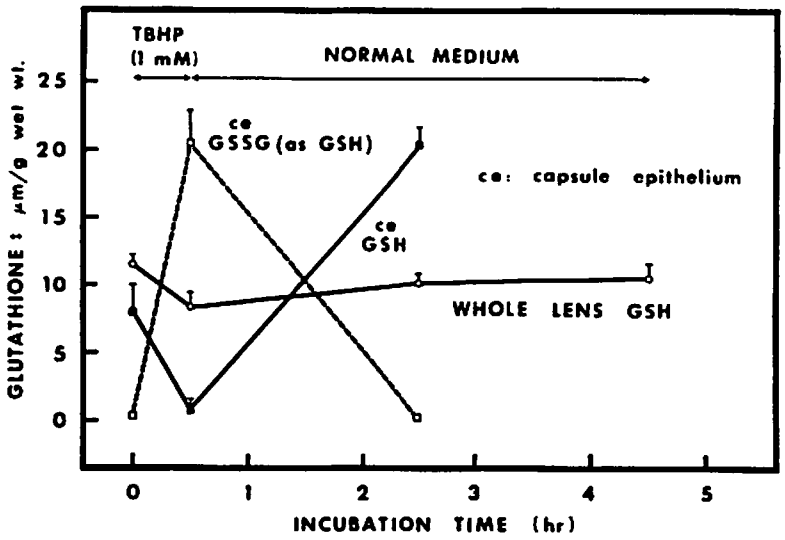


FIGURE 2. The Regeneration of GSH in Whole Lens and Capsule Epithelium of Rabbit Lenses Previously Exposed to t-butyl hydroperoxide (TBHP). Lenses were preincubated for 30 min in Tyrode's medium containing 1 mM TBHP and then incubated for additional time in normal medium. Separate lenses were used for glutathione assays in whole lens and capsule epithelium. Results are expressed as means ± S.D. for at least four experiments. Reprinted by permission from Ref. 12.

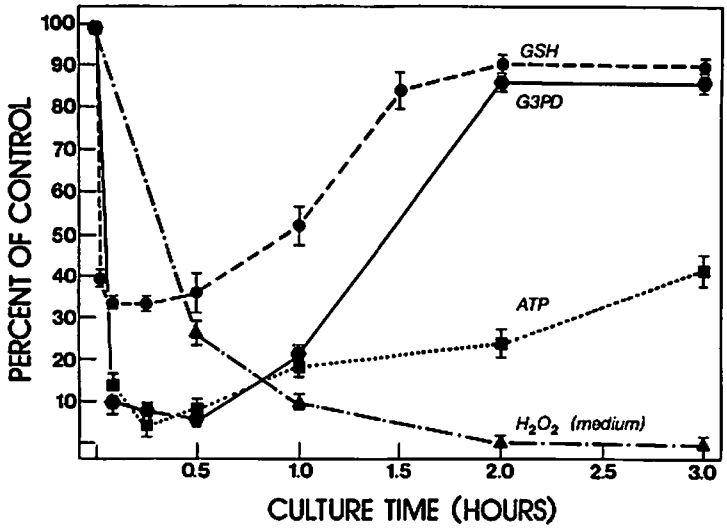


FIGURE 3. Exposure of $8x10^5$ Normal Rabbit Lens Epithelial Cells to a Single Pulse of 0.5 mM $H_2O_2$ in 5 ml MEM at 37°C. The activity of glyceraldehyde 3-phosphate dehydrogenase and levels of GSH and ATP were determined in the cells at various times following the pulse of $H_2O_2$. The concentration of $H_2O_2$ in the medium is expressed as a percent of the initial level of 0.5 mM. Results are expressed as means ± S.E. for at least four experiments. Reprinted by permission from Ref. 19.

(Fig. 3). Previous studies in our laboratory have indicated that lens epithelial GSH levels must drop more than 60% of normal before changes in epithelial functions are elicited (12). In the experiment of Fig. 3, through the activity of catalase and glutathione peroxidase in the cells (19), the level of $H_2O_2$ in the

culture medium is rapidly brought down to zero and, at the same time, cellular glutathione redox cycle activity reduces GSSG and returns GSH and also G3PD activity to nearly 90% of their control values. ATP concentration, initially depleted after $H_2O_2$ exposure, also soon begins to climb back to its normal level. Thus, antioxidant protection in the lens epithelial cells is able to effectively defend against $H_2O_2$-induced cell death.

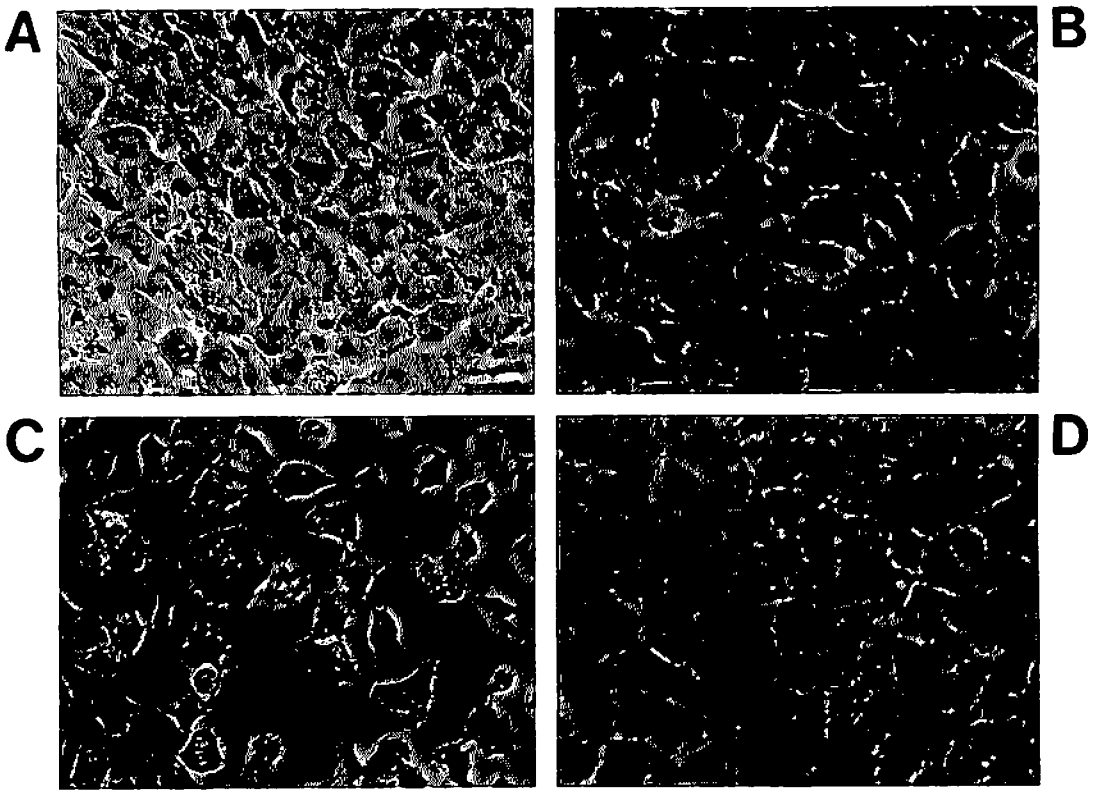


FIGURE 4. Photomicrographs of Cultured Rabbit Lens Epithelial Cells. Cells (800,000) were cultured in MEM **(A)**; MEM + 0.5 mM $H_2O_2$ **(B)**; CDNB/$H_2O_2$ **(C)**; or CDNB/nPG/$H_2O_2$ **(D)**. Six hr later, cells were cultured in MEM containing 8% rabbit serum and photographed after 7 days of culture. Note the protection afforded by nPG (identical results were obtained for deferoxamine or TEMPOL in place of nPG). (x140). Reprinted by permission from Ref. 20.

Recently, in collaboration with John Reddan, we conducted experiments similar to those described in Fig. 3; however, in this study, some of the cells were pretreated with either buthionine sulfoximine (BSO) or chlorodinitrobenzene (CDNB) to decrease the initial level of GSH in the cells by 85% prior to exposure to $H_2O_2$ (20). When this was done, there was, surprisingly, no difference observed in the rates of $H_2O_2$-detoxification by normal and GSH-depleted cells. However, if after exposure of the cells to $H_2O_2$, when they were cultured normally in 8% serum for 7 days, whereas the normal cells treated with $H_2O_2$ appeared healthy (Fig. 4B), the GSH-depleted cells challenged with $H_2O_2$ were severely damaged and nearly all dead (Fig. 4C). This result indicated an important role for a high level of GSH in the lens cells to defend against some type of oxidative challenge; however, the protection was independent of the cells' ability to detoxify $H_2O_2$. An additional finding was that a brief pretreatment of the cells with either n-propyl gallate (nPG) or deferoxamine, or co-treatment of the cells with the nitroxide free radical

TEMPOL, were able to substitute for GSH in the BSO- or CDNB-treated cells and prevent $H_2O_2$-induced cell death (Fig. 4D). Each of these three compounds is known to be effective in preventing the formation of hydroxyl radical which can be generated by $H_2O_2$-induced Fenton-type reactions (21,22). Thus, it was shown that high levels of GSH may be needed in lens epithelial cells to protect against hydroxyl radical-induced cell death. GSH has been demonstrated to be a good scavenger of hydroxyl radical, but only when it is present at high levels of at least 5 mM (23). Normal rabbit lens epithelial cells, in contrast to GSH-depleted cells, are able to effectively scavenge hydroxyl radical and prevent cell death since they possess >20 mM levels of GSH.

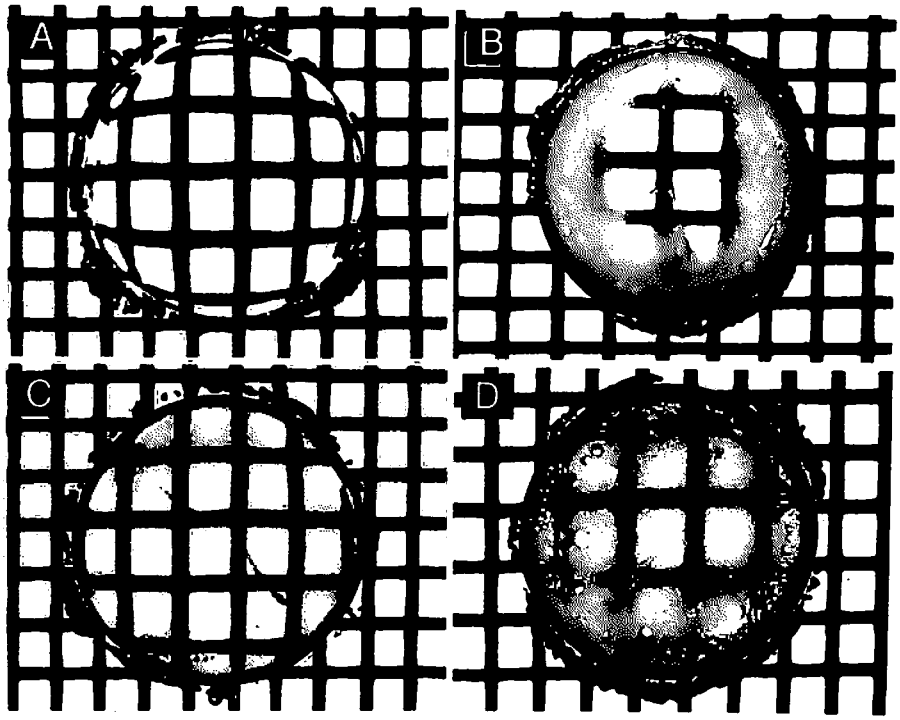


FIGURE 5. Photographs of Rabbit Lenses Cultured under Various Conditions. Lenses were cultured for 3 hr in the presence or absence of glucose and 1 mM DHA at 20°C in 5 ml Tyrode's medium with 10 mM Hepes, pH 7.1. Lenses treated with BCNU were preexposed to 0.5 mM BCNU at 37°C in TC-199 medium for 3 hr. **(A)** - glucose; **(B)** - glucose, + DHA; **(C)** + glucose, + DHA; **(D)** BCNU/DHA, +glucose. See text for explanation of abbreviations. Reprinted by permission from Ref. 24.

In addition to the reduction of active species of oxygen in the lens epithelium, GSH has also been shown to non-enzymatically reduce dehydroascorbic acid (DHA) (24). When dog lens epithelial cells were challenged with increasing concentrations of DHA in the absence of glucose (glucose was left out of the medium in order to prevent the reduction of GSSG formed within the cells), there was a progressive loss of GSH in the cells and an increase in the level of GSSG as the external concentration of DHA was increased from 0.05 to 2.0 mM. At each concentration of DHA studied, the amount of GSSG measured in the cells was nearly equal to the amount of GSH lost. No evidence could be found for the participation of an enzyme in the reduction of DHA by GSH in the lens cells (24). The fact that DHA can be damaging to the lens was shown by exposing cultured rabbit lenses to DHA, either in the absence of glucose (Fig. 5B), or after inhibiting the activity of glutathione reductase with 1,3-bis (2-

chloroethyl)-1-nitrosourea (BCNU) (25) (Fig. 5D) which produced severe loss of transparency. These studies showed that even an essential antioxidant such as ascorbic acid can be harmful to the lens if it is not maintained in the reduced form.

What are the lens epithelial targets that are protected from oxidative damage by high levels of GSH? They appear to include certain proteins containing key -SH groups essential for normal lens epithelial function such as Na/K-ATPase, certain cytoskeletal proteins, and proteins associated with normal membrane permeability. To investigate the role of GSH in protecting the lens against oxidative damage to cation transport, rabbit lenses were pretreated with BCNU to inhibit the activity of glutathione reductase in the capsule-epithelium by 71% and then exposed to a maintained level of 0.05 mM $H_2O_2$. Figure 6A shows that when this was done, a 30% decrease in the activity of Na/K-ATPase was observed in the lenses after 3 hr of $H_2O_2$ treatment; the loss of activity was not significantly reversed following 6 hr of culture in normal medium. The same protocol also produced a much faster efflux of $^{86}Rb$ from the experimental lenses (Fig. 6B; $t_{1/2}$=25 hr for controls and 7 hr for BCNU/$H_2O_2$-treated lenses); in this case, a reversal of $H_2O_2$-induced effects on membrane permeability was observed when the lenses were returned to a normal medium. The percentage of GSSG present in the lens capsule epithelium decreased from 72% at the end of the BCNU/$H_2O_2$ treatment to 26% at the end of the 6 hr of culture in normal medium (26), suggesting that reduction of key -SH groups may have been associated with the recovery in permeability. The BCNU/$H_2O_2$ treatment also resulted in a significant loss of lens transparency in the form of distinct vacuoles around the periphery of the lens (26). To investigate whether depletion of GSH alone in the lens epithelium could inactivate Na/K-ATPase, rabbit lenses were pretreated for 30 min with CDNB to conjugate 70% of the GSH in the capsule epithelium (27). This treatment had no effect on the level of Na/K-ATPase activity, nor did a 4 hr exposure to a maintained level of 0.05 mM $H_2O_2$. However, a combined CDNB/$H_2O_2$ challenge produced a 60% drop in Na/K-ATPase activity, again demonstrating the importance of GSH in protecting this enzyme from oxidative inactivation.

The role of GSH in protecting the lens epithelial cytoskeleton was shown by pretreating rabbit lens epithelial cells with BCNU and then exposing them to 0.025 mM $H_2O_2$ maintained for one hr. This treatment damaged the cells, producing numerous blebs on the cell surface (28). TEM micrographs shown in Fig. 7 indicate a disruption of organization of the apical layer of the microfilaments corre-

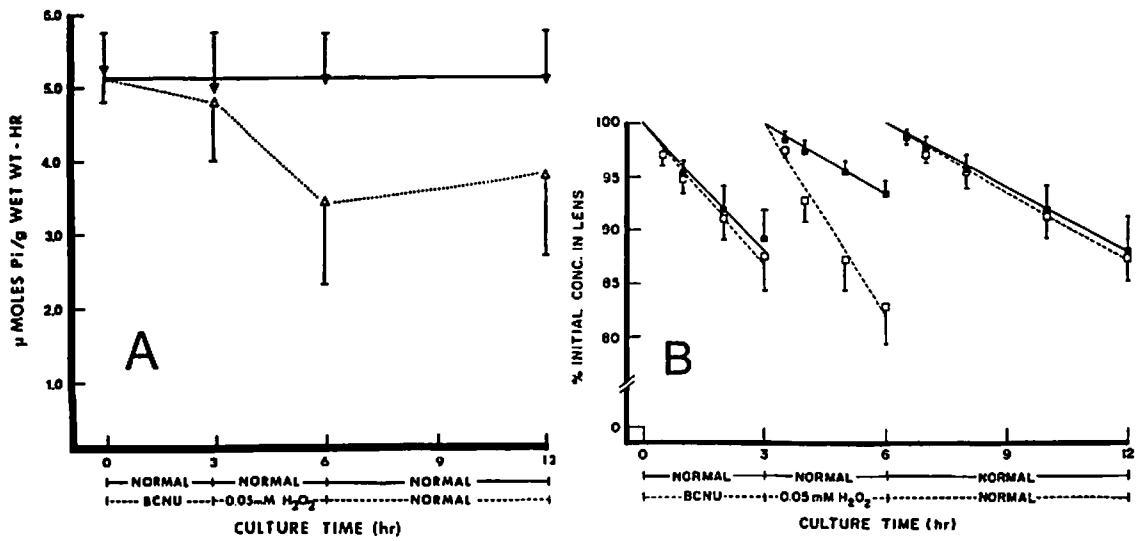


FIGURE 6. Effect of BCNU/$H_2O_2$ Treatment on Rabbit Lens Cation Transport Parameters Under Various Conditions. The level of BCNU employed was 0.5 mM. Contralateral lenses were used as controls. Results are expressed as means ± S.D. for 5-8 experiments. **Solid symbols**: control; **open symbols**: experimental. **A**: Na/K-ATPase activity. **B**: The efflux of $^{86}Rb$. Lenses were preloaded overnight with $^{86}Rb$ and then treated as indicated in the figure. The efflux of $^{86}Rb$ into the culture medium was measured at various times. Reprinted by permission from Ref. 26.

sponding to bleb formation; the microfilaments are discontinuous, and many cells show an aggregation or clumping of microfilamentous-like materials. The BCNU/$H_2O_2$-induced blebs appeared to be caused by a weakening of the microfilament layer located beneath the apical surface of the cell. In later experiments, it was found that formation of $H_2O_2$-induced blebs in rabbit lens epithelial cells could be prevented with the nitroxide free radical compound TEMPOL (22), thus providing evidence for protection by GSH against hydroxyl radical damage to epithelial cytoskeletal proteins.

In studies with transgenic mice with deficient and elevated levels of glutathione peroxidase (GSHPx) activity, the important role of the glutathione redox cycle was demonstrated in protecting the lens epithelium against $H_2O_2$-induced damage, including the formation of single-strand breaks in epithelial DNA (29). When lenses were exposed to a 25 μM concentration of $H_2O_2$, DNA strand breaks in the transgenic mouse lens (having a 5-fold higher activity of GSHPx) were 40% of those in a normal lens treated with $H_2O_2$, while the extent of DNA damage in the peroxide-treated GSHPx knockout mouse was 5 times greater than that of the GSHPx-rich transgenic mouse lens (29). Thus, in addition to protecting the lens epithelium against oxidative attack on cytoskeletal proteins and on proteins associated with cation transport, GSH can also prevent oxidative damage to the genome.

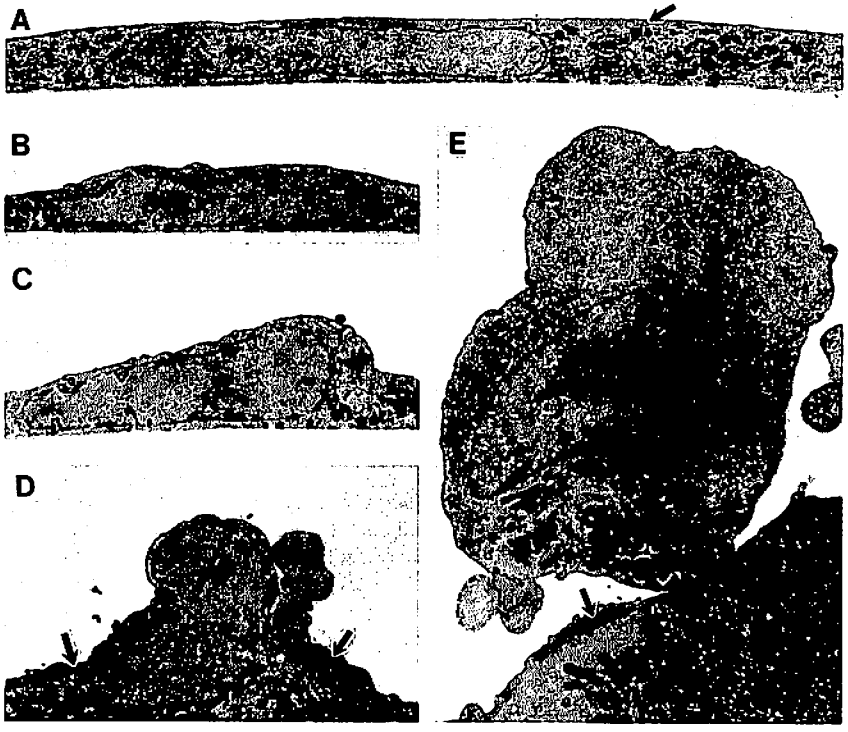


FIGURE 7. TEM Micrographs of Cultured Lens Epithelium. x6400. **(A)** Cells cultured for 3 hr in normal medium. A layer of microfilaments is visible just under the cell membrane **(arrow)**. **(B-E)** Cells incubated with 0.025 mM $H_2O_2$ for 1 hr after BCNU treatment in high **(B-D)** and low **(E)** cell density areas. The progressive reorganization of the microfilament sheath **(arrows)** corresponds to bleb formation. The damage is more severe in cells from the low cell density regions of the culture dish. Reprinted with permission from Ref. 28.

## Consequences of a Low Lens Nuclear GSH Level

In contrast to the lens epithelium and cortex, the nuclear region of the lens possesses low levels of GSH and related antioxidant activity. GSH concentrations are 80-90% lower in the nucleus of the rabbit and guinea pig lens compared to the cortex (10,30); age exacerbates this difference as has been shown

for lenses of the guinea pig, rat and human (30-32). While the ratio of GSH to target protein -SH groups in the lens cortex is nearly one to one, this proportion drops to one to ten in the nucleus (33). Recently, Sweeney et al. have provided evidence for an age-induced barrier in the human lens, preventing the migration of GSH from the cortex to the nucleus and possibly initiating the formation of human senile cataract (34). The activity of the key HMPS enzyme, glucose 6-phosphate dehydrogenase, is 90% lower in the nucleus of the rabbit lens, compared to the cortex, and glutathione reductase activity and NADPH levels are 50% lower (35). It has long been thought that this low level of antioxidant protection in the lens nucleus makes this region especially vulnerable to oxidative damage and cataract (31). A substantial amount of evidence has been gathered to show that the development of human senile nuclear cataract is, indeed, associated with oxidative damage (reviewed in Ref. 11).

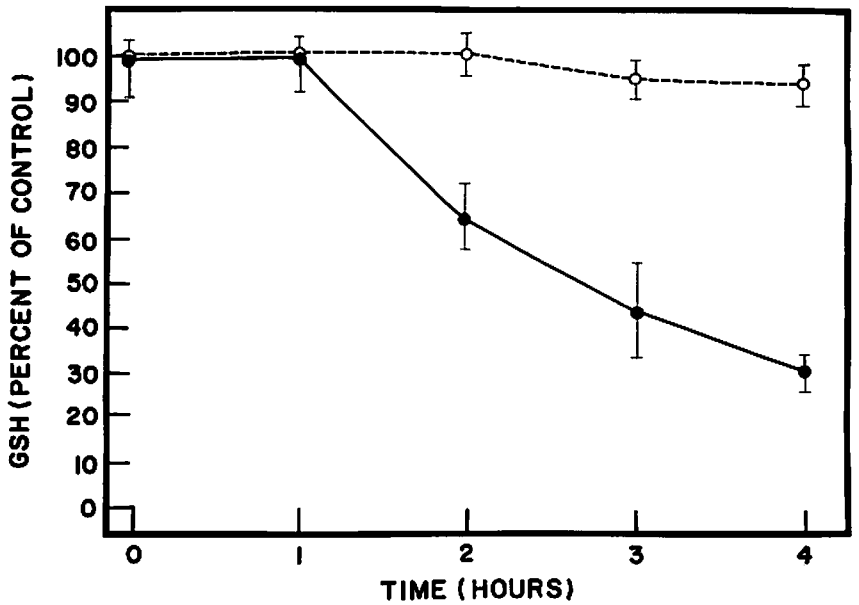


FIGURE 8. The Effect of Hyperbaric $O_2$ on the Levels of GSH in Different Regions of the Cultured Rabbit Lens Expressed as Percent of Control. Lenses were cultured for various times at 37°C under 8 atm of 99% $O_2$ (remainder $CO_2$). Contralateral lenses were used as controls for each experimental point and were cultured at 1 atm of air. **Open symbols**: epithelium plus superficial cortex (40% of lens weight); **closed symbols**: inner nucleus (20% of lens weight). Results are expressed as means ± S.E. for four to eight experiments. Control GSH values (μmol/g) were 16.0 ± 2.0 and 2.3 ± 1.0 for cortex and nucleus, respectively (n=25). Reprinted with permission from Ref. 10.

To investigate the vulnerability of the lens nucleus to oxidative stress, cultured rabbit lenses were treated with hyperbaric $O_2$ (HBO), using lenses cultured in 1 atm air as controls. When lenses were exposed to 8 atm of 99% $O_2$ for 2 hr, the level of GSH decreased by 35% in the nucleus of the lens with no effect observed on the concentration of the tripeptide in the superficial cortex (Fig. 8). Similarly, after 4 hr of exposure to $O_2$, while there was only a slight (<10%) decrease of GSH level in the superficial cortex of the lens, the loss of GSH in the nucleus amounted to 70%. Table 1 shows that the epithelium of the lens was able to withstand 3 hr of exposure to 50 atm of $O_2$ with no loss of GSH or accumulation of GSSG; only at 75 and 100 atm of $O_2$ did a detectable level of GSSG appear in the epithelium. Similar *in vitro* studies involving the treatment of rabbit and bovine lenses with HBO showed that disulfide-crosslinked crystallins and glyceraldehyde 3-phosphate dehydrogenase, as well as oxidatively-modified aldose reductase, formed first and to a greater extent in the nucleus compared to the cortex (33,36). In separate experiments, it was demonstrated that GSSG accumulated in the fibers of HBO-treated rabbit

lenses, and activity of the HMPS increased as GSH was regenerated in the lenses (10). In addition, HBO-treatment of GSH solutions produced substantial amounts of $H_2O_2$, the formation of which could be blocked by addition of the metal-chelating agent EDTA. When these data are viewed overall, they suggest that molecular $O_2$ can lead to metal-catalyzed autoxidation of GSH (and, most likely, also ascorbic acid) in the lens and to the formation of potentially damaging $H_2O_2$. These *in vitro* studies show that the nuclear region of the lens is significantly less able to defend against $O_2$-induced stress, compared to the epithelium and the cortex.

TABLE 1.
Effect of Hyperbaric Oxygen on the Levels of Reduced and Oxidized Glutathione in the Capsule-epithelium of Cultured Rabbit Lenses

| $O_2$ Pressure (atm) | Glutathione (nmol/ce) | | GSSG |
|---|---|---|---|
| | GSH | GSSG (as GSH) | (% of total) |
| 8 | 26 | N.D. | 0 |
| 15 | 43 | N.D. | 0 |
| 30 | 31 | N.D. | 0 |
| 50 | 41 ± 8 | N.D. | 0 |
| 75 | 31 ± 8 | 14 ± 4 | 32 |
| 100 | 12 | 9 | 43 |

Lenses cultured for 3 hr at 37°C under various pressures of 99% $O_2$ (remainder $CO_2$). Analysis of GSH and GSSG was done with an amino acid analyzer. Results are expressed as either means of two experiments or means ± S.D. for four experiments. Each capsule epithelium (ce) weighed approximately 4 mg. N.D. = not detectable. Reprinted with permission from Ref. 10.

We next wanted to learn whether the susceptibility of the lens nucleus to molecular oxygen observed *in vitro* could also be demonstrated with the use of an HBO *in vivo* experimental animal model. An earlier study had reported that human patients treated therapeutically with HBO developed increased lens nuclear light scattering (NLS) and nuclear cataracts (37). Older guinea pigs (initially 18 months of age) were treated three times a week with 2.5 atm of 100% $O_2$ for 2.5 hr periods. Fig. 9 shows that treatment of the animals 30 and 51 times with HBO produced an increased level of lens NLS and an enlargement of the lens nucleus compared to age-matched controls. Analysis by TEM indicated that the increased NLS was associated with morphological alterations to experimental lens nuclear membranes that included distention of the intercellular spaces between fiber cells, especially at the Y junctions formed between three neighboring fibers (11). These changes were similar to those which had been reported for human immature nuclear cataracts (38). No morphological effects were observed in the cortex or epithelium of the HBO experimental lenses. GSH levels were found to decrease by about 30% in the lens nucleus after 30 treatments of the animal with HBO; at this point, the concentration of GSH in the experimental nucleus was nearly 90% lower than that present in the cortex of the same lens (11). In contrast to the lowered levels of GSH in the lens nuclei of the $O_2$-treated animals, concentrations of protein-thiol mixed disulfides were found to be elevated. Data are shown in Fig. 10 for protein-bound cysteine (PSSC), but similar results were observed for protein-bound glutathione (PSSG) and protein bound γ-glutamyl cysteine (11). After 30 and 65 treatments of the guinea pigs with HBO, PSSC concentrations in the lens nucleus increased to five times the control values; levels of the compound in the experimental cortex were ten times lower than those in the nucleus of the same lens (Fig. 10). The concentrations of nuclear PSSC and PSSG observed as a result of HBO-treatment were remarkably high, in the same order of magnitude as the normal level of nuclear GSH, and coincided with the appearance of increased NLS.

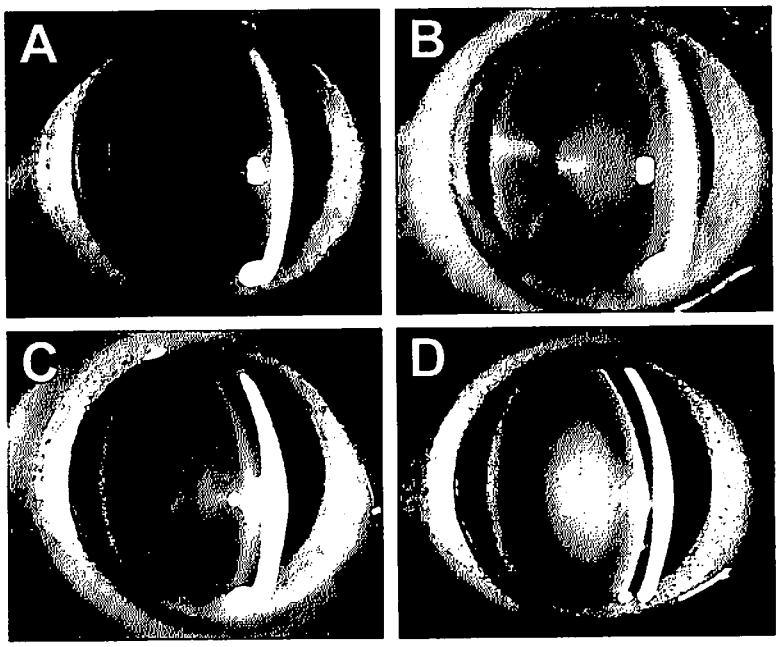


FIGURE 9. Slit-lamp Biomicroscopy Photographs of Guinea Pig Eyes. **(A)** 20.5 month-old control; **(B)** 20.5 month-old after 30 treatments with hyperbaric $O_2$; **(C)** 22.25 month-old control; **(D)** 22.25 month-old after 51 treatments with hyperbaric $O_2$. Reprinted with permission from Ref. 11.

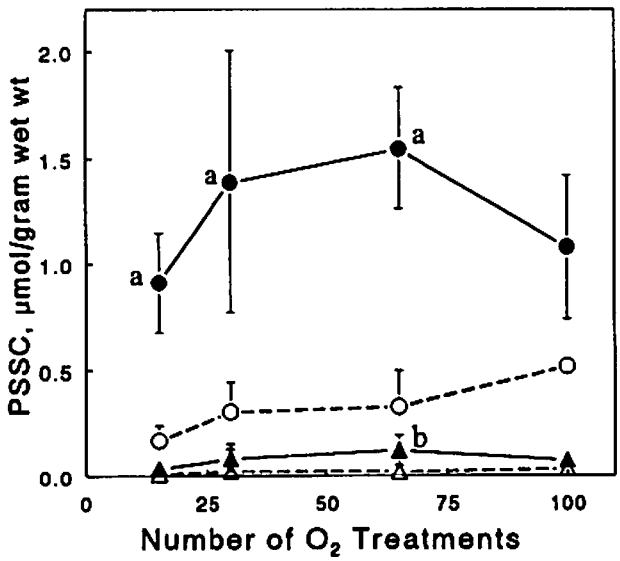


FIGURE 10. Concentrations of Protein-bound Cysteine in the Lens Cortex and Nucleus (Total Protein Fractions) of Guinea Pigs Receiving Different Numbers of Treatments with Hyperbaric Oxygen. Ages of control and experimental animals were 19, 20, 24 and 30 months after 15, 30, 65 and 100 $O_2$ treatments, respectively. Error bars indicate S.D. Numbers of lenses analyzed were 12-13, 9-10, 4-8 and 2-3 for 15, 30, 65 and 100 $O_2$ treatments, respectively (each lens was from a separate animal). **Closed circle**: nucleus, $O_2$-treated; **open circle**: nucleus, control; **closed triangle**: cortex, $O_2$-treated; **open triangle**: cortex, control. a: $P<0.001$ compared to control; b: $P<0.01$ compared to control. Reprinted with permission from Ref. 11.

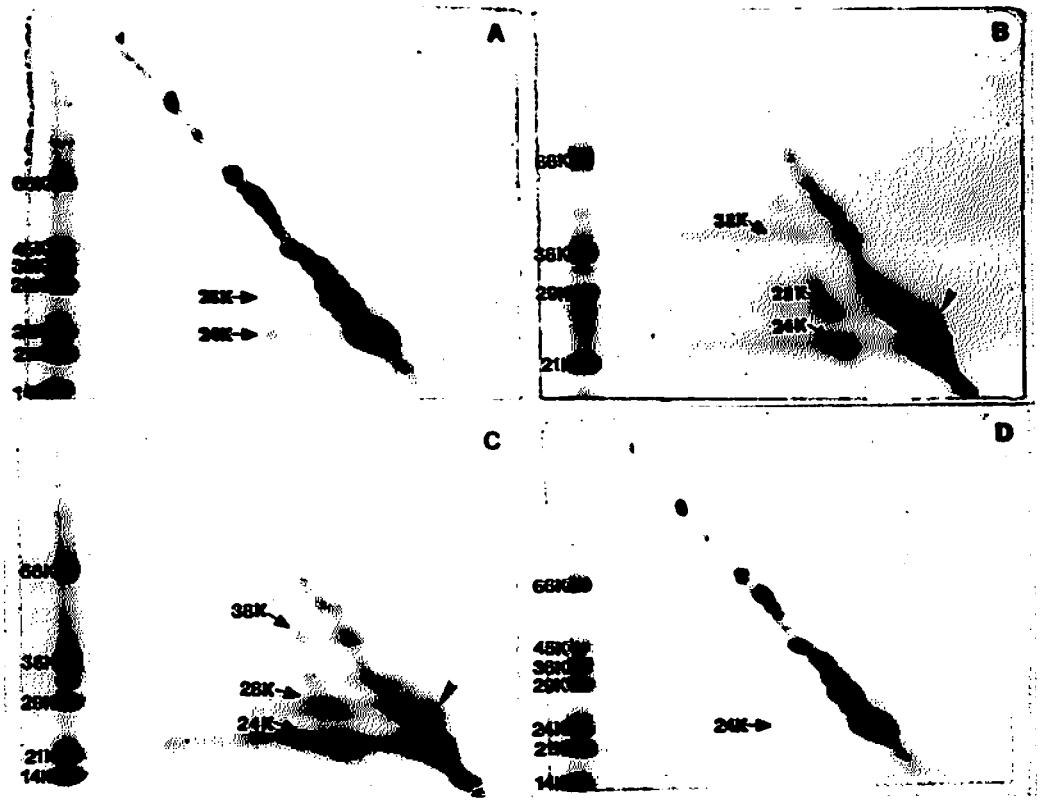


FIGURE 11. Coomassie Blue-stained Two-dimensional Diagonal SDS-PAGE Patterns of Urea-insoluble Protein Fractions of Guinea Pig Lens Cortex and Nucleus. Samples of 120 µg protein were run on tube gels in the first dimension without prior treatment with mercaptoethanol. The tube gels were incubated for 30 min in buffer containing mercaptoethanol and applied on the second dimension. The presence of off-diagonal spots **(arrows)** indicates disulfide-crosslinked proteins. Also note the protein which appears to the right of the diagonal **(arrowheads)**. **(A)** Lens nucleus from a normal 1 month-old animal. **(B)** Lens nucleus from a normal 20 month-old animal. **(C)** Lens nucleus from a 20 month-old animal after 30 treatments with hyperbaric $O_2$. **(D)** Lens cortex from a 20 month-old animal after 30 treatments with hyperbaric $O_2$. Reprinted with permission from Ref. 11.

Human cataracts have also been reported to possess elevated levels of the three disulfide compounds (39,40). The source of the cysteine to form PSSC in the lens nucleus is unclear at this time, since levels of free cysteine in the lens are usually very low (41).

Similar to the results for protein-thiol mixed disulfides, levels of protein disulfide (PSSP) also increased preferentially in the lens nucleus of HBO-treated animals, compared to the cortex. The amounts of PSSP in the lens nuclei of untreated, control guinea pigs increased as the animals aged from 1 to 20 months-old (Fig. 11 A,B); 30 treatments of the animals with HBO significantly accelerated this age-related increase in PSSP level in the lens nucleus (Fig. 11C), but not in the cortex (Fig. 11D). In separate studies, loss of cytoskeletal proteins and MIP26, related apparently to disulfide crosslinking of the proteins, was also found to be accelerated in the lens nucleus, but not in the cortex of older guinea pigs treated with HBO (30). However, when younger, two month-old guinea pigs were exposed to HBO, only α- and β-tubulins in the lens nucleus were found to be susceptible to $O_2$-induced loss, apparently as a result of their unusually high sulfhydryl content and increased sensitivity to oxidation (30). The diminished effect of HBO on cytoskeletal proteins and MIP26 in the two-month old lens nucleus compared to the 20 month-old is possibly linked to the higher level of GSH in the younger tissue (5 vs. 3 µmol/g wet wt., Ref. 30).

X-ray-induced cataract in the rabbit offers another example of the increased susceptibility of the lens nucleus to oxidative stress. Seven weeks after X-ray of the animal, a period that is one week prior to the formation of a mature, completely opaque cataract, the nucleus of the lens, but not the cortex, contains a significantly higher level of disulfide-crosslinked protein, compared to the controls (42). Similar to the other models which have been discussed, this observation appears to be associated with a relatively low level of GSH in the experimental lens nucleus (1.2 μmol/g compared to 3.5 μmol/g in the experimental cortex for the X-ray model). Results of the X-ray study, as well as those for the HBO *in vitro* and *in vivo* models, suggest that some critical level of GSH of approximately 1 mM exists in the lens nucleus, below which nuclear proteins are able to form disulfide-crosslinks.

Recent studies, published thus far only as Abstracts (43,44), have used a UVA light *in vivo* model and the glutathione peroxidase knockout mouse as further demonstration of the significant morphological effects and increased light scatter which can occur in the nucleus of the lens of an experimental animal, without appearing in the cortex.

## SUMMARY

The described studies have shown that a healthy lens epithelium and cortex are driven to maintain a reduced environment in order to ensure normal epithelial function and lens transparency. Oxidants such as $H_2O_2$, DHA and hydroxyl radical are readily detoxified by a high level of lens epithelial GSH, with resultant GSSG effectively reduced back to GSH by an active glutathione redox cycle. Targets protected in the lens epithelium by GSH include proteins which contain key -SH groups essential for epithelial function such as Na/K-ATPase, certain cytoskeletal proteins and proteins associated with the maintenance of normal membrane permeability. In contrast to the outer regions of the lens, the lens nucleus is relatively deficient in GSH and related antioxidant activity, and this differential becomes more evident as the tissue ages. The susceptibility of the lens nucleus to oxidative damage and loss of transparency has been demonstrated by a number of *in vivo* experimental animal models including hyperbaric $O_2$, X-ray, UVA light and the glutathione peroxidase knockout mouse. A need exists for development of therapeutic agents to slow the age-related loss of antioxidant activity in the nucleus of the human lens to delay the onset of senile nuclear cataract.

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Reprint Requests: Frank J. Giblin, Ph.D.
Eye Research Institute
Oakland University
412 Dodge Hall
Rochester, Michigan 48309-4401 U.S.A.
Email: giblin@oakland.edu