MINIREVIEW

Ocular Oxidants and Antioxidant Protection (44250)

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Abstract. **Oxidative damage and antioxidant protection in ocular tissues has not been reviewed recently. Metabolism in the eye is of increasing interest because the organ is highly susceptible to damage by sunlight, oxygen, various chemicals, and pollutants. Interest is expected to increase because of an aging Western world population and a continued depletion of stratospheric ozone. Hydrogen peroxide is discussed because it is both a byproduct and a source of free radical reactions and is normally present in the aqueous humor. The metabolism of reactive oxygen species by enzymes, nutrients, pigments, and low molecular weight scavengers is evaluated. Ascorbic acid, because of its high concentration in the eye, is thought to be a primary substrate in ocular protection; progress in determining the mechanisms by which it is recycled and maintained in the useful, reduced state is discussed. Recent information is included about antioxidants not previously known to be present in the eye, and some importance is placed on the properties of the vitreous humor and tear fluid because of the previous lack of emphasis on these.** [P.S.E.B.M. 1998, Vol 217]

The sense of vision would not be possible if the eye had an opaque protective dermal covering like the rest of the body. The eye is therefore a unique organ because it is relatively unprotected and is constantly exposed to radiation, atmospheric oxygen, environmental chemicals, and physical abrasion. The retina has an additional threat from conversion of radiation to neural impulses transmitted to the brain. Each of these factors results in generation of reactive oxygen species (ROS) or various free radical species ($R^{\cdot}$) thought to contribute to ocular damage and disease (1, 2). Growing concern exists because of reports from geophysical scientists that the earth's stratospheric ozone is being depleted. This depletion will allow additional solar radiation to reach the earth's surface and put more stress on human vision.

Pertinent, current research being conducted on the role of oxidants and antioxidants in the eye has not been widely reviewed. The term "antioxidant" appears only in one chapter of Adler's text entitled *Physiology of the Eye* (3), and biochemistry texts of the eye also give relatively little coverage to oxidants and antioxidants and their role in the eye (4). The most recent review emphasizing primarily free radicals and ocular disease appeared in 1994 (5). Instead the most contemporary reviews are associated with the role of oxidants and antioxidants in cancer (6) and in diseases of the nervous system (7–11).

Oxidants and sensitizers known to be present in eyes of diurnal animal species include hydrogen peroxide (H_2O_2), singlet oxygen, superoxide anion, hydroxyl radical, riboflavin, tryptophan and its oxidation products, and NAD^+ (12). Natural protective components (4, 13) include water soluble antioxidants (e.g., vitamin C, cysteine, glutathione, uric acid, pyruvate, and tyrosine) some of which are considered herein and several not mentioned, including lipid-soluble

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antioxidants (e.g., tocopherols and retinols), specific enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase), and metal-binding proteins (e.g., transferrin, ceruloplasmin, and the albumins). An authoritative review on antioxidants specific to the retinal pigmented epithelium is available (14), and reviews of the epidemiology of antioxidant consumption in eye diseases such as age-related cataract and macular degeneration (15–17) have also appeared recently. The present review will highlight recent research findings regarding ROS and water soluble antioxidants in ocular tissues. Reactive oxygen species is an inclusive term used to describe both oxidants (e.g., H_2O_2) and free radical species (e.g., superoxide, hydroxyl radical).

Reactive Oxygen Species Found in the Eye

Predominant sources of ROS associated with ocular tissues include 1) radiation from exposure to sunlight; 2) superoxide and other oxygen radicals generated from normal mitochondrial respiration and reduction of oxygen to water; and 3) H_2O_2 produced from a variety of intracellular and extracellular metabolic reactions. The actions and effects of these ROS are not readily separable because most often they are found together and exhibit an interactive and even a "cascade-like" relationship (e.g., production of one may lead to production of another). For example, oxygen radicals and H_2O_2 often result from exposure to radiation or ultraviolet light. However, experimental investigations were usually performed by exposing tissues to one of the three and therefore, the following literature review is organized by studies in which one of the three was the primary parameter being manipulated.

Radiation. As a photon of radiation is brought to chemical rest, sufficient energy is released to the immediate environment to strip an electron from a local molecule and result in a free radical species ($R^{\cdot}$). A free radical species can pass its extra unpaired electron to other molecules, so that a cascade of reactions occurs resulting in direct damage to anatomic and physiologic components of the eye such as lens cellular membranes (18). In studies on photooxidative stress (19, 20), isolated retinal pigmented epithelial cells were incubated with ^{14}C-ascorbic acid and exposed to a visible laser. The amount of ^{14}C-ascorbate oxidized to ^{14}C-dehydroascorbate was found to be proportional to the amount of radiation delivered, and the oxidation of ascorbate was suggested to result from free radicals formed when ocular pigments (especially melanin), were illuminated with light. Rozanowska (21) confirmed recently that melanin was the key retinal pigment responsible for the photosensitized oxidation of exogenous ascorbate. Data indicated that photoinduced melanin radicals oxidized ascorbate, and the reduced melanin was subsequently reoxidized by oxygen with the formation of superoxide and H_2O_2. Spector and coworkers (22) have shown by *in vitro* electron spin resonance (ESR) studies that riboflavin in the presence of light stimulates oxygen consumption (oxidation) and formation of ESR-detectable ascorbyl semiquinone radicals causing lens epithelial cytotoxicity *in vitro*. The oxidation of ascorbate was confirmed to be dependent upon photosensitizer concentration and could be inhibited by superoxide dismutase, catalase, and albumin (22).

Oxidation products of ascorbic acid rapidly glycate proteins and produce protein-bound, advanced glycation end products that can absorb ultraviolet-A (UVA) light and cause the photolytic oxidation of proteins, which is suggested to be mediated by the formation of ROS, including superoxide anion, H_2O_2, and singlet oxygen (23, 24). The relative rate of ascorbate and glucose oxidation has been compared under conditions used for glycation reactions *in vitro* and by UVA-generated oxygen radicals using human lens sensitizers (25). In this study, superoxide anion and singlet oxygen were identified as the principal oxidants of ascorbate, and the authors concluded that ascorbate may be the primary glycating agent in aging normal lenses (25). A recent study (26) was conducted to study nitrite-induced oxidation of thiols and reduced glutathione (GSH) in corneal epithelial extracts. Oxidation of GSH in the presence of nitrite was minimal in the dark, but exposure of GSH to UVA (365 nm) light in the presence of nitrite substantially accelerated the oxidation so that less than 10% of the original GSH remained at the end of 20 min. Ascorbate was found to be effective in preventing thiol oxidation, suggesting the possibility of preventing nitrogen oxide–based smog irritation to the eye by a physiologically compatible antioxidant. Based on these results, the authors (26) further suggested that nitrite is a potent phototoxicant with possible pathophysiological implications to the external eye tissues.

The suggestion has been made that the aqueous humor acts as a UV-filter protecting the structures behind itself. This hypothesis has been evaluated further by use of spectrophotometry and spectrofluorimetry (27). Three different aspects of the protective mechanism have been unveiled: absorption, fluorescence quenching, and wavelength transformation. The high ascorbate values in the aqueous humor were suggested to play a key role in all three because aqueous ascorbate was shown to increase absorption and suppress fluorescence of radiation below about 310 nm wavelength. In addition, as a consequence of ascorbate quenching, fluorescence emission to the UVA range (320–400 nm) was substantially reduced (27), supporting the hypothesis above and that ascorbate may be a key player in this proposed role for the aqueous humor.

Oxygen. Atmospheric oxygen is a threat to the health of all aerobic organisms and in the human, the potential for damage is particularly great in the retina and cornea. Controversy exists as to the source of anterior chamber oxygen, specifically whether the chamber receives oxygen from the atmosphere *via* diffusion through the cornea or whether the anterior iris vasculature is the major source. Barr and Silver (28) suggested that the ambient environment is the main source for oxygen, based on their finding of only a slight change in pO_2 after an animal dies. Even though the avascular cornea appears to depend on a continuous flow of

aqueous humor for supply of nutrients, the anterior surface of the cornea is a substantial distance (~5 μm) from the aqueous humor, considering that the route of delivery would be by membrane transport across the corneal endothelium followed by simple diffusion through the thick stroma. Thus, in the human, the corneal epithelium is potentially one of the cell types most vulnerable to damage. Moreover, potential damage can be delivered instantaneously and locally through heat, light, chemicals, or physical abrasion; therefore, protection by endogenous antioxidants is essential. In a recent set of experiments (29), the question of whether the isolated intact epithelial bilayer, derived from rabbit ciliary processes, transports ascorbic acid and to what degree the transport rate *in vitro* corresponds to the *in vivo* process was addressed. Results indicated that transfer of ascorbic acid across the bilayer occurred at a rate required to maintain the ordinary millimolar concentration of ascorbic acid found *in vivo* in the aqueous humor (29). This study would support the anterior iris vasculature as a potential source of oxygen and antioxidant nutrients to the cornea.

Retinal cells have a rapid rate of oxygen utilization; thus, the retina is another potential site of oxygen-induced injury or disease. In addition, the rod/cone outer segments have high levels of polyunsaturated fatty acids (4) that are preferred substrates for peroxidation reactions. Lowered levels of antioxidant vitamins E and C and consequent accumulation of oxygen and lipid free radical species have been suggested as an explanation for the inflammation, neovascularization, and retinal pathology observed in patients with Eales' disease (30).

Whatever the predominant oxygen source to lenses, the lens normally remains clear despite exposure to ultraviolet radiation and endogenous H_2O_2. In the presence of trace amounts of free transition metals and photosensitizers (e.g., riboflavin and tryptophan), ascorbate readily reacts with oxygen yielding H_2O_2, which could damage lens cell membranes or crystallines (31). Eaton (32) proposed that the real antioxidant function of ascorbate in aqueous and vitreous humors is in the conversion of oxygen to H_2O, which produces a metabolically sustained anaerobiosis. He further suggested that if this occurs, "nature may have preinvented the process of canning, wherein food (or in this case, the lens) is preserved by a combination of sterility and anoxia" (32).

Hydrogen Peroxide. In addition to radiation and oxygen radicals, H_2O_2 or lipoperoxides are potential key oxidants in the aqueous humor. Hydrogen peroxide is present in concentrations ranging from about 0.025 to 0.070 m*M* in humans (4). Although some controversy exists, researchers generally agree that cataract is associated with changes in levels of H_2O_2 in the aqueous humor. The controversy appears to center on the direction of the change. In human aqueous humor, H_2O_2 has been found by separate investigators (33, 34) at concentrations 20-fold higher than normal in patients with age-related cataract. However, at least one researcher has observed a reduced level of H_2O_2 in aqueous humor obtained from patients with cataract (35).

Hydrogen peroxide *in vitro* can arise from a nonenzymatic reaction between ascorbate, riboflavin, and light or unbound trace metals, which might constitute a pro-oxidant role for ascorbate (4). In support of this idea, Giblin (36) observed that the concentration of H_2O_2 in aqueous humor of the rabbit and guinea pig was directly related to the concentration of ascorbate. This relationship was investigated (36, 37) by injecting ascorbate (2 × 50-mg injections, 3 hr apart) intraperitoneal (ip) into rabbits, later quantifying and correlating aqueous humor ascorbate and H_2O_2 using the dichlorophenol-indophenol (DHCIP) method of analysis. Unfortunately, earlier techniques, such as the DHCIP method, which assay H_2O_2 and ascorbate or other suspected reducing agent(s), are now considered inaccurate (33). Furthermore, injecting a concentrated bolus of ascorbate ip, far from the target organ to be assayed, does not simulate the normal physiologic state. However, in spite of these shortcomings, the results of these experiments by separate investigators suggest a relationship between levels of ascorbate and H_2O_2, and have been compelling enough to give credence and a sense of finality to the theory that ascorbate can act as a pro-oxidant *in vivo* and act as a source of H_2O_2 under certain conditions. In additional support of this theory, results of a recent study (38), conducted to investigate the effect of ascorbate on oxidative injury induced by t-butyl hydroperoxide in cultured porcine retinal pigment epithelial (RPE) cells, indicated that ascorbate was toxic to RPE. However, in the presence of catalase pretreatment, ascorbate provided protection against the oxidative injury induced by t-butyl hydroperoxide (38) suggesting that H_2O_2 and possible formation of hydroxyl radicals were involved in the toxicity.

A number of investigators (39–41) demonstrated that, with inhibition of glutathione synthesis, the concentration of H_2O_2 in aqueous humor remained constant or decreased. Further, with inhibition of glutathione reductase, the aqueous humor concentrations of ascorbate and H_2O_2 did not change. In experiments by Costaraides (41), H_2O_2 was injected intracamerally through the cornea of pigmented rabbits along with inhibitors of glutathione synthase (buthionine sulfoxamine—BSO), glutathione reductase (1,3-bis-(2-chloroethyl)-1-nitrosourea—BCNU), or catalase (3 amino triazole—3AT). Both BSO and 3AT separately prolonged the time required to eliminate exogenously added H_2O_2 from the anterior chamber. BSO resulted in a 77% increase in elimination time after 10 μl of 10 m*M* H_2O_2 was injected intracamerally, while suppression of catalase activity with 3AT was somewhat less effective (40% increase in time for elimination of H_2O_2), having more influence at higher concentrations of injected H_2O_2. The role of ascorbate in this proposed redox system requires further clarification. However, a dynamic equilibrium was suggested to exist for H_2O_2 through its production by ascorbate oxidation and its

subsequent elimination by tissue hydroperoxidases and chemical reaction with GSH (42).

Babizhayev *et al.* (43) and Giblin *et al.* (40) found lens epithelial tissue *in vitro* to be a potent degrader of H_2O_2. Encircling anterior segment tissues comprise a metabolically active compartment that manifests excellent H_2O_2 detoxification systems, including catalase (high K_m) and glutathione peroxidase (low K_m). Glutathione peroxidase and catalase have been found in most ocular tissues including lens, corneal epithelium and endothelium, iris, ciliary body, and retina (44, 45).

In studies where the purpose was to analyze the activities of catalase, glutathione peroxidase, and superoxide dismutase in lenses isolated from Rhesus monkey and subsequently maintained in culture, results indicated that after exposure to H_2O_2, both catalase and glutathione peroxidase activity were decreased, and only superoxide dismutase was stimulated above control lens levels (46). In another investigation (47) in which researchers examined the contribution of glutathione peroxidase in degrading H_2O_2 in lens preparations, the consistent overall conclusion was that glutathione peroxidase and catalase function together, but when glutathione peroxidase is knocked out or glutathione reductase is inhibited, catalase can provide protection from H_2O_2 stress, suggesting that glutathione peroxidase was not required for normal function. Conclusions from other work regarding H_2O_2 and ascorbate (42) indicate that ascorbate might act as a scavenger of free radicals with H_2O_2 being a necessary by-product, but one that is less reactive than the original oxidants. In a recent study (31), the oxidative effect of H_2O_2, ascorbate, and glucose in the presence of transition metals was studied in bovine lens membranes. Incubation of lens with H_2O_2 and metal, ascorbate and metal, or glucose and metal resulted in a significant increase in thiobarbituric acid–reactive substances in the membranes. The oxidation was found to be mediated by hydroxyl radicals and in all experiments Cu(II) ions exhibited a higher efficiency compared to Fe(II) ions (31).

Based upon present knowledge from *in vitro* experiments (42) and from theory (32), the mechanism of ascorbate's action in the eye appears to be to degrade H_2O_2 and consume oxygen, thus driving the anterior chamber of the eye in a hypoxic direction. If this hypothesis is correct, ascorbate, under normal circumstances, does not act as a pro-oxidant but as an antioxidant. The effect *in vivo* might be largely dependent on pO_2, light, ascorbate concentration, and the presence of free metals or photosensitizers, but minimally dependent on the endogenous low concentration of GSH in the aqueous humor or that provided by the contiguous circulation.

"Pro-oxidants" in the Eye

No oxidant or effective antioxidant is considered to function alone in the body. The roles of copper and iron are particularly interesting because of their interaction with ascorbate and other antioxidant molecules. In plasma, iron is bound to the transport protein transferrin, which is considered to have a scavenging function preventing Fe^{2+} from participating in the Fenton reaction and subsequently forming free radicals leading to lipid peroxidation. Unlike ascorbate, iron is present at a much lower concentration in the aqueous and vitreous humors than in the plasma of different animal species with little variation among species and no obvious difference between nocturnal and diurnal animals (48). Superoxide anion can reduce iron to the ferrous state, which can then react with H_2O_2 resulting in formation of the highly reactive hydroxyl radical. Dimethylthiourea has been found to be a potent scavenger of toxic oxygen metabolites such as the hydroxyl radical. Furthermore, intraperitoneal administration of dimethylthiourca to New Zealand white rabbits significantly reduced iris hyperemia and other inflammatory responses to subsequent intravitreal injection of *Escherichia coli* endotoxin (49). Iron binding capacity of proteins is as important in the eye as it is elsewhere in the body, and the binding is responsive to oxidative challenge. In the rabbit eye, both free iron and total-iron-binding capacity increased to peak levels 24 hr after intravitreal injection (10 ng) of endotoxin and gradually declined to baseline levels within 3 weeks (50). Ascorbate has been shown to increase the concentration of the iron storage protein, ferritin, in cultured lens epithelial cells; the ascorbate-induced increase in ferritin concentration was suggested to be due mainly to an increase in ferritin synthesis at the translational level (51). The ability of ascorbic acid to increase ferritin concentration in lens epithelial cells was implied to be an additional protective mechanism for ascorbate. The importance of ferritin to normal lens function was underscored by the recent finding (52) that humans with a dominantly inherited abnormality in ferritin synthesis exhibit early bilateral cataracts. Binding capacity of metals in ocular tissues requires additional research.

As mentioned earlier, the oxidation products of ascorbic acid have been shown to react with lens proteins to form advanced glycation end products capable of generating ROS when irradiated with UVA light (23, 53). L-Threose is the most active of the oxidation products (23, 54); it rapidly glycates proteins and produces protein-bound, advanced glycation end products. These end products can absorb UV light and cause the photolytic oxidation of proteins (53), which is mediated by the formation of ROS, including superoxide anion, H_2O_2, and singlet oxygen (24). L-Threose detoxification to L-threitol by aldose reductase has been evaluated in the lens; however, further studies on the role of this enzyme in preventing toxicity due to degradation products of ascorbate are in progress (55, 56). The effects of high hexose levels on the redox equilibrium between ascorbate and dehydroascorbate (DHAA) was examined in cultured rat lenses (57) by exposing the cultured lenses to high galactose levels and then incubating them with ^{14}C-labeled ascorbate, DHAA, or diketogulonic acid (DKG). Results indicated that when lenses were incubated with ascorbate or DHAA, an elevated level of the degradation product, DKG,

was detected in galactose-exposed lenses. Ascorbate uptake was enhanced, but regeneration of ascorbate from DHAA in galactose-treated lenses was impaired (57). In addition, the galactose-treated lenses showed enhanced permeability to DKG demonstrating that profound abnormalities in ascorbate metabolism exist in lenses exposed to a high sugar environment (57).

Ascorbate has also been shown to inhibit cell growth. In a recent study (58), a corneal epithelial cell line was used to study the effect of ascorbic acid on proliferation. The addition of ascorbate at concentrations ranging from 0.5–3.0 m*M* was shown to lead to a 40% dose-dependent inhibition of proliferation in corneal epithelial cell cultures after 6 days, with an IC_{50} of 0.7 m*M* ascorbic acid. Based on these findings, the authors concluded that the local application of ascorbate for treatment of chemical burns of the cornea with no stromal involvement should be subjected to a critical reassessment (58).

Metabolism and Scavengers of ROS

Several scavengers of ROS are present in the eye and can be grouped into nutrients (e.g., vitamins A, E, C), antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase, metallothionein), several low molecular weight substances (e.g., uric acid, GSH, tyrosine), and pigments (e.g., xanthophylls, melanin, and tryptophan oxidation products). The present review ties together some of the material presented in an earlier examination of ocular ascorbic acid transport and metabolism (59) with more recent developments.

Mechanisms of Protection. Delivery of substances from the plasma *via* the aqueous humor to the anterior cornea could take as long as 5 hr, given the slow rate of aqueous humor secretion and the considerable diffusion barrier of the corneal stroma. A more immediate source for protection and repair of the eye's anterior surface might come through lacrimal secretions. The advantage of this source is that the cornea can be reached within seconds following an insult. A recent study used high performance liquid chromatography (HPLC) and electrochemical detection to demonstrate the presence of five water-soluble antioxidants, ascorbic acid, cysteine, GSH, uric acid and tyrosine, in human tear fluid collected at "basal" and stimulated flow rates (59a). The mechanism and glandular source of these antioxidants have not been identified, although ascorbic acid was previously found to be taken up and kept from oxidation in the lacrimal gland of pigs (60). Delivery of plasma components through the iris-ciliary body into the aqueous humor (29) and vitreous humor (61) also occurs.

The aqueous humor itself does not appear to sustain permanent damage by oxidative stress; any deterioration of significant components thought to have a role in antioxidant protection appears to be reversible upon return to optimal conditions. However, long-term insult results in loss of antioxidants along with tissue damage (62).

In spite of potential pro-oxidant activities, ascorbate continues to confirm its importance as the most effective, versatile, protective water-soluble antioxidant found in the eye. In a study by Stoyanovsky, *et al.* (63), results demonstrated that endogenous ascorbate in the retina and in rod outer segments was able to protect endogenous α-tocopherol against oxidation induced by UV-irradiation apparently by reducing the phenoxyl radical of α-tocopherol. Authors also demonstrated that in the absence of ascorbate, neither endogenous nor exogenously added GSH was efficient in protecting α-tocopherol against oxidation, and GSH did not substantially enhance the protective effect of ascorbate against α-tocopherol oxidation. In addition, exogenous dihydrolipoic acid was able to enhance the protective effect of ascorbate by reduction of DHAA (63). Besides ascorbate, protection is provided against oxidative stress by enzymes such as superoxide dismutase, glutathione peroxidase, and catalase. Babizhayev (64) showed recently that human cataractous lenses displayed decreased activity of glutathione peroxidase and increased levels of conjugated dienes, iodometric and TBA-reactive substances when incubated with liposomal membranes. These oxidation products were demonstrated to be decreased in the presence of free radical scavengers and antioxidant enzymes including EDTA, superoxide dismutase, chelated iron, and catalase (64). The results of these types of studies continue to support the idea that antioxidants and antioxidant enzymes are extremely important in providing protection to ocular tissues in the face of oxidative stress.

Humans must acquire vitamin C from dietary sources, and levels of the vitamin are compromised in a variety of diseases (for a recent review, see (65)). Therefore the effect of supplementation or dietary restriction is of interest. In studies in which the effects of feeding a normal diet were compared with the effects of a diet restricted by 40% relative to control animals, no change in ascorbate levels in the lens was observed, but dietary restriction was significantly correlated with delayed cataract progression (66, 67). Diabetes induced an increase in oxidative stress and a decrease in ATPase activity in the retina, both of which were shown to be inhibited by dietary supplementation with vitamins C and E (68). In additional studies by the same group, exogenous vitamin C and E supplementation was shown to alleviate the changes observed in certain retinal antioxidant defense enzymes including SOD and enzymes of the glutathione redox cycle that were significantly impaired in diabetes and experimental galactosemia (55). The effects of an aldose reductase inhibitor (TAT) on GSH and ascorbic acid levels was studied in streptozotocin diabetic rats (69). Treatment with TAT suppressed the increased malonodialdehyde levels observed in lens, aqueous humor, and serum and normalized the decreased levels of GSH and ascorbic acid observed in both galactose and streptozotocin diabetes rats (69).

Besides ascorbate, DHAA has been shown to possess vitamin C–like activities as well as protect the lens against oxidative stress and cataract formation in the rat lens *in vitro*

(70). Some experimental results suggest that the beneficial effects of DHAA may be attributed to its property of undergoing peroxidative decarboxylation and of O^{-}_{2} (radical anion) scavenging (56). Many researchers contend that the protective effects of DHAA are due to ascorbate, which results from the *in vivo* enzymatic or chemical reduction of DHAA (65).

Ocular Metabolism of Vitamin C. Ascorbic acid is suggested to offer significant antioxidant protection to the eye by scavenging ROS (59, 71). Ascorbate accumulates in ocular tissues of diurnal animal species at a concentration (0.5–2 m*M*) several times higher than that found in the plasma (0.03–0.2) (72) and in most ocular tissues, is higher than the concentrations of other water soluble antioxidants. A model for the role of ascorbate in the eye is suggested in Figure 1. Reactive oxygen species are generated from a variety of sources as indicated earlier. Reaction 1 shows the interaction of ascorbate with free radicals (R˙) generated from radiation, oxygen metabolism, carcinogen metabolism, leukocyte metabolism, or other sources. Neutralization of ROS by ascorbate results in generation of the ascorbyl free radical (Reaction 1). Pairs of ascorbyl free radicals may spontaneously decay (half-life in msec) with the loss of an unpaired electron (e^{-}) resulting in net production of dehydroascorbate (Reaction 2). In some tissues, ascorbyl free radicals may be converted to ascorbate by ascorbyl free radical reductase (Reaction 3) (73). Besides a chemical reduction by GSH (74), conversion of DHAA to ascorbate is believed to occur in most tissues *via* an enzymatic process involving GSII and/or NADPH and one or more DHAA

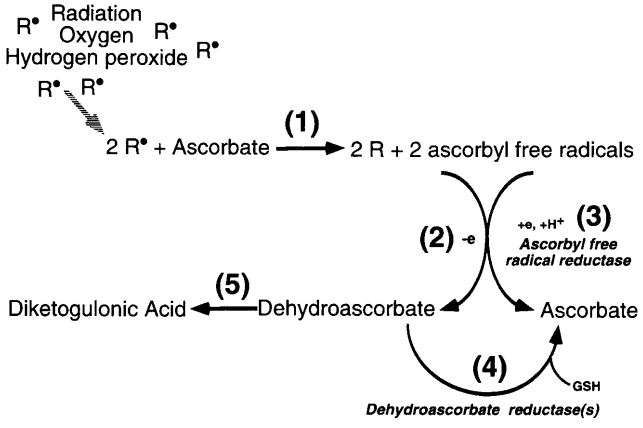

Figure 1. Ascorbic acid as a scavenger of free radicals in the eye. Free radicals (R•) are generated from sources such as radiation (sunlight), mitochondrial oxygen metabolism, and hydrogen peroxide. Reaction (1): Ascorbate interacts with free radicals resulting in the production of the ascorbyl free radical. Reaction (2): Pairs of ascorbyl free radicals may spontaneously decay (half-life in msec) with the loss of an unpaired electron (e^{-}) resulting in net production of dehydroascorbate. Reaction (3): Ascorbyl free radicals may be also be converted to ascorbate by ascorbyl free radical reductase. Reaction (4): Dehydroascorbate may be converted to ascorbate chemically by GSH or in an enzymatic process involving glutathione and/or NADPH and one or more dehydroascorbate reductases. Reaction (5): In the absence of reducing equivalents, dehydroascorbate may decay in a biologically irreversible process to form diketogulonic acid.

reductases (75–78) (Reaction 4). On the other hand, at the pH and temperature of most body fluids, DHAA decays with a half-life of about 6 min (79) in a biologically irreversible process to form diketogulonic acid (Reaction 5).

Accumulated evidence suggests that a dysfunction in ascorbic acid metabolism may have a role in the etiology of a number of diseases including cataract formation (80, 81) and diabetes (81, 82). Diabetes is associated with decreased ascorbate and increased DHAA levels in a variety of mammalian tissues including the eye (83–85). Abnormally high levels of DHAA have been shown to be disruptive to cell membranes (86, 87) and diabetogenic (88). The mechanism by which ascorbate is maintained in its reduced form is of particular importance with regard to ocular tissues, which appear to have a need for high levels of the reduced vitamin.

The oxidative state of ocular tissues is of much current interest in that ascorbate has been shown to protect the aqueous humor (27), the retina (89), and the lens (70, 90) of the eye from excessive light damage. On the other hand, metabolic products of ascorbate metabolism and/or degradation have been linked to cataract formation and protein crosslinking (23, 53, 91). Some evidence indicates that GSH alone has a major role in mammalian tissues for maintaining ascorbate in its useful reduced form (74). In addition, substantial evidence supports an enzymatic reduction by a variety of proteins (75–78, 92). A general but comprehensive review on the metabolism of vitamin C in health and disease (65) and a review of the role of nutrition and antioxidants in delaying or preventing cataract formation in humans are available (90).

At least three bovine ocular tissues (iris-ciliary body, cornea, and retinal pigmented epithelial cells) exhibit DHAA-reducing activity. This activity was studied extensively in a partially purified extract of iris-ciliary body (93). The activity was pH dependent, linear with increasing protein concentration, and inactivated by either high temperature or trypsin digestion (93). Significantly, the enzymatic process exhibits saturation kinetics, suggesting the presence of an enzymatic mechanism for DHAA reduction to ascorbic acid. The apparent K_ms for both DHAA (0.24 m*M*) and GSH (0.5 m*M*) were within the physiological range of concentrations of these compounds. Two recent reports describe the purification and characterization from rat liver of two proteins having DHAA reductase activity. These reports support evidence for the dependency of DHAA reduction on GSH and/or NADPH (77, 78). Whether or not these proteins are present in ocular tissues has yet to be determined.

Other investigators have suggested the direct involvement of *known* enzymes (enzymes characterized primarily for other metabolic activities), including thioltransferase, in the recycling of ascorbate (75). Thioltransferase activity was identified and partially purified from ocular tissue (lens) for the first time (94); the activity depended on the presence of GSH, glutathione reductase (GR), and NADPH to reduce the disulfide bond in a synthetic substrate, hy-

droxyl ethyl disulfide (HEDS). Maximum activity was obtained in a pH 7.4 phosphate buffer at 30°C. Thioltransferase may be an important antioxidant component in the lens along with ascorbate, GSH, and glutathione reductase in protecting the vulnerable lens proteins against oxidative damage. Semidehydroascorbate reductase (also called ascorbyl free radical reductase or monodehydroascorbate reductase) is thought to be a membrane associated protein that catalyzes the combination and subsequent reduction of the ascorbyl free radical to form ascorbate (see Figure 1) (73). Ascorbyl free radical reductase activity has been separated from a soluble fraction of human lens cortex and shown to be decreased in activity in cataractous lenses (95–97). Evidence for the presence of DHAA reductase activity (98) in the lens has recently been presented. The activity ($K_{m_{DHAA}}$ 0.45 m*M*) was found to be restricted primarily to the mitochondrial fraction isolated from the cortex-epithelium of the tissue and was not detectable in the cytosolic fraction (98). In contrast to these research results, evidence also exists in which reduction of DHAA was shown to be only linked to the glutathione redox cycle by a nonenzymatic interaction between GSH and DHAA in lens epithelium (99).

Importance of Glutathione. Glutathione is the most prevalent cellular thiol and in many cells GSH accounts for more than 90% of the total nonprotein sulfur (100). Concentrations as high as 12–15 m*M* have been found in lens tissue (65) where it maintains lens protein thiols in the reduced state, protects membrane -SH groups, and is a cofactor in the detoxification of H_2O_2 (101). Decreases in GSH to levels 10%–90% (102, 103) below normal (65) have been observed in the lens of aging rats with cataract (104, 105) and in the retina (106) and lens (107) of diabetic rats. The decrease has been suggested to be linked to a diminution in the GSH redox system. This system is believed to protect ocular tissues from damage by low concentrations of H_2O_2 whereas catalase is suggested to protect ocular tissues when higher concentrations of H_2O_2 are present (37, 41). Ascorbate has been shown to spare GSH in GSH deficiency (37, 108); and, in ascorbate deficient guinea pigs, treatment with GSH delays the appearance of scurvy (109).

The biochemistry of protein-glutathione mixed disulfide formation was examined by C-13-NMR spectroscopic measurements of glutathione oxidative metabolism in intact rabbit lenses maintained in organ culture (110). These experiments provided insight into the role of the cellular glutathione redox-couple, GSH/GSSG, in maintaining reduced protein thiol groups. Results indicated that protein-glutathione adduct formation may function as a mechanism for modulating the glutathione redox state under conditions of oxidative stress in ocular tissue. In addition, the results demonstrated the feasibility of direct chemical reduction of protein-glutathione disulfide bonds *in vivo,* which may reflect a mechanism for the inhibition of disulfide-linked light scattering protein aggregate formation (110).

A significantly lower content of sulfhydryl proteins was found in the lens and vitreous humors of diabetic patients than in those of nondiabetic or control subjects (84). Moreover, an increased formation of protein-bound free sulfhydryls and carbonyl proteins, indices of oxidative damage to proteins, was noted in diabetic patients. In addition, glutathione peroxidase activity and ascorbic acid levels were found to be significantly decreased in the lens of these diabetic patients, especially in the presence of retinal damage. These results (84) were suggested to be an indication of an altered protein redox status in subjects affected by diabetes mellitus.

A significant age-related decrease in GSH levels as well as depression of γ-glutamyl-cysteine synthetase activity are factors believed to render the aged lens more susceptible to oxidative stress and, therefore, to cataractogenesis. In an attempt to find a means of attenuating the inevitable age-related decrease, acetyl thioester and ethyl ester were evaluated for their GSH-enhancing activity in cultured human and rat lenses *in vitro* using an assay that measured the incorporation of ^{14}C-glycine into lens GSH (111). Results indicated that both were effective in raising GSH levels suggesting that these compounds may have potential as anticataract agents (111).

In addition to the synthesis and recycling of GSH, transport of circulating GSH may be important in protecting ocular tissues. Besides the rat canalicular GSH transporter (RcGshT) (112) present in the lens (113), results from another study (114) provide strong evidence for the presence of a Na^+-dependent GSH transporter in the lens epithelium distinct from the RcGshT. This transporter may mediate concentrative, basolateral uptake of aqueous GSH consistent with *in situ* eye perfusion studies (114). Transport of GSH was also studied (115) at the basolateral side of the lens epithelium by using an *in situ* vascular eye perfusion technique in guinea pigs with rapid sampling to ensure detection of initial rate of uptake. The authors concluded that circulating GSH is probably a major source for epithelial GSH under physiologic conditions, and transport of GSH at the basolateral side of the lens epithelium is mediated by a mechanism distinct from RcGshT (115).

The relationship of the ascorbic acid and the glutathione redox cycle to the reduction of the oxidized product of ascorbic acid, DHAA, is extremely important and has been studied in dog lens epithelium (99). Treatment of lens cells with a pharmacological dose of DHAA (1 m*M*) for 0.5–3 hr in the absence of glucose resulted in 60%–100% oxidation of GSH and distinct morphologic changes. The addition of glucose, required for the reduction of glutathione disulfide (GSSG) *via* the glutathione redox cycle, prevented these effects and allowed nearly immediate recovery of GSH after DHAA exposure in the absence of glucose. In these experiments, the reduction of DHAA in lens epithelium was shown to be linked to the glutathione redox cycle by a nonenzymatic interaction between GSH and DHAA, and no evidence of DHAA reductase activity was observed (99).

Other Areas of Interest

Uric Acid as an Ocular Antioxidant. Uric acid is a substance recently recognized to be important in free radical metabolism. A contemporary review (116) presents a persuasive case that uric acid is as effective an antioxidant as ascorbic acid, and urate is present in the blood at a relatively high concentration (300 μM). The comparative physiology is interesting because animal species other than primates degrade uric acid and excrete the waste produce, allantoin. Humans conserve uric acid by a reabsorptive process in the kidney and although detailed information is available about the role of uric acid in the heart (117), much less attention has been paid to ocular uric acid. Human aqueous humor was obtained at the time of surgery and analyzed for uric acid by HPLC (118), results indicated that the uric acid concentration in some glaucomatous eyes was higher than normal. Regardless of what caused the elevated uric acid in these eyes, chronic exposure of trabecular tissues to a high uric acid concentration might have an influence on proteoglycans and alter resistance to aqueous drainage.

Role of Tyrosine in the Eye. The electrochemically active amino acid tyrosine is of second highest concentration compared to other aqueous humor water soluble antioxidants in humans (119). Tyrosine is suggested to be a hydroxyl radical scavenger, singlet oxygen quencher, and weak photosensitizer (120). These properties are distinct from its well-known roles as an enzyme cofactor in DNA synthesis, an amino acid in signal transduction, and a precursor for melanin and catecholamines. Little is known regarding tyrosine in the eye, however, in other tissues, superoxide ($O_2^{\cdot-}$)/nitric oxide ($NO^{\cdot}$) generate phenoxyl radicals when reacting with tyrosine. The role of tyrosine as a pro-oxidant in $O2^{\cdot-}$/$NO^{\cdot}$-initiated LDL oxidation was tested; but surprisingly, when LDL was exposed to $O2^{\cdot-}$/$NO,^{\cdot}$ tyrosine exerted a strong inhibitory effect on $O2^{\cdot-}$/$NO^{\cdot}$-initiated LDL oxidation as measured by TBARS formation and alteration in electrophoretic mobility of LDL and tyrosine was also able to protect human endothelial cells from the cytotoxic effect of $O2^{\cdot-}$/$NO^{\cdot}$ (121). Whether tyrosine can exert a similar protective effect from $O2^{\cdot-}$/$NO^{\cdot}$ cytotoxicity in the eye is not known. Its properties have been evaluated in synthetic aqueous humor, where it tends to act as an oxidant in agreement with empirical observations and one electron reduction potential-thermodynamic predictions (121). In that the aqueous humor/plasma ratio of tyrosine is 1.74 in rabbits, 1.26 in monkeys, and 1.84 in human (3), tyrosine appears not to undergo active transport from the ciliary epithelium.

Vitreous Humor and Antioxidant Protection. The composition and mechanism of formation of the vitreous humor have received less research attention than other fluids and tissues of the eye. The first detailed account of antioxidants in the animal vitreous humor has demonstrated significant concentrations of cysteine, ascorbic acid, GSH, uric acid and tyrosine in one or more animal species (61). These data allow a speculative conclusion of the difference between diurnal animal species (rabbit and bovine) versus a nocturnal species (rat). The low level of ascorbic acid in rat vitreous humor relative to diurnal animals is similar to the corresponding information on aqueous humor (72) and might have a similar rationale, (i.e., that vitamin C is not as necessary in eyes of nocturnal animals because of its proposed function of protection against UV radiation). However, the high levels of GSH, uric acid, and tyrosine in rat vitreous humor have not found a ready justification based on comparable teleology.

Tear Fluid. Little has been published about the content of antioxidants in tear fluid. Tear fluid from healthy human subjects was recently collected either into borosilicate glass tubing or by absorption onto Schirmer strips (59a). HPLC with electrochemical detection was used to evaluate antioxidants and related compounds. Tear fluid collected at basal flow rates contained cysteine (83 μM), ascorbic acid (811 μM), GSH (128 μM), uric acid (260 μM) and tyrosine (33 μM). Also consistently present on the chromatogram was a prominent peak that was not identified. When the flow rate of tear fluid was stimulated by inhalation of ammonia fumes, the levels of ascorbic acid and cysteine decreased much more than those of uric acid or the unidentified component (59a). These results suggest that tears derived from distinct glandular sources may have an antioxidant content related to a specific protective role.

Summary

The eye is constantly exposed to oxidative stress. Antioxidants in the eye comprise a system of interrelated nutrients, enzymes, pigments, and low molecular weight scavengers. Because ascorbic acid is concentrated in ocular tissues, it is considered important in protecting against radiation, oxygen radicals, and exposure to hydrogen peroxide and other oxidants. Additional antioxidants such as uric acid, GSH, and the amino acid, tyrosine, may also play an important protective role.

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