Published in final edited form as:

Eye Contact Lens. 2011 July ; 37(4): 233–245. doi:10.1097/ICL.0b013e31821ec4f2.

Role of UV Irradiation and Oxidative Stress in Cataract Formation. Medical Prevention by Nutritional Antioxidants and Metabolic Agonists

Shambhu Dayal Varma, M.Sc., Ph.D. FARVO.^{1,*}, Svitlana Kovtun, M.D.¹, and Kavita Rajeev Hegde, M.D., Ph.D.²

¹Department of Ophthalmology and Visual Sciences, University of Maryland School of Medicine, Baltimore, MD 21201

²Department of Natural Sciences, Coppin State University, Baltimore, MD 21216

Abstract

Purpose—Cataract is a significant cause of visual disability with relatively high incidence. It has been proposed that such high incidence is related to oxidative stress induced by continued intraocular penetration of light and consequent photochemical generation of reactive oxygen species such as superoxide and singlet oxygen and their derivatization to other oxidants such as hydrogen peroxide and hydroxyl radical. The latter two can also interact to generate singlet oxygen by Haber-Weis reaction. It has been proposed that in addition to the endogenous enzymatic antioxidant enzymes, the process can be inhibited by many nutritional and metabolic oxyradical scavengers such as ascorbate, vitamin E, pyruvate and xanthine alkaloids such as caffeine.

Methods—Initial verification of the hypothesis has been done primarily by rat and mouse lens organ culture studies under ambient as well as UV light irradiation and determining the effect of such irradiation on its physiology in terms of its efficiency of active membrane transport activity and the levels of certain metabolites such as GSH and ATP as well as in terms of apoptotic cell death. In vivo studies on the possible prevention of oxidative stress and cataract formation has been done by administering pyruvate and caffeine orally in drinking water as well as by their topical application using diabetic and galactosemic animal models.

Results—Photosensitized damage to lens has been found to be significantly prevented by ascorbate and pyruvate caused by exposure to visible as well as UVA. Caffeine has been found to be effective against UVA as well as UVB. Oral or topical application of pyruvate has been found to inhibit the formation of cataracts induced by diabetes as well as galactosemia. Caffeine has also been found to inhibit cataract induced by sodium selenite as well as high levels of galactose. Studies with diabetes are in progress.

Conclusion—Various in vitro and in vivo studies summarized in the review strongly support the hypothesis that the light penetration into the eye is a significant contributory factor in the genesis of cataracts. The major effect is through photochemical generation of reactive oxygen species and consequent oxidative stress to the tissue. The results demonstrate that this can be averted by use of

*Corresponding Author: Shambhu D. Varma, M.Sc., Ph.D. FARVO Professor and Director of Research Ophthalmology and Visual Sciences Professor, Biochemistry and Molecular Biology University of Maryland School of Medicine MSTF 5-77, 10 South Pine Street Baltimore, MD 21201 Phone: 410-706-3395; Fax: 410-706-7057 svarm001@umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

various antioxidants administered preferably by topical route. That they will be so effective is strongly suggested by the effectiveness of pyruvate and caffeine administered topically to diabetic and galactosemic animals.

Keywords

Oxidative stress; antioxidants; UV irradiation; cataracts

Introduction

Oxidative stress has now been well accepted to be involved in the pathogenesis of many aging diseases such as atherosclerosis, myocardial infarction, Alzheimer's disease, Parkinson's disease, schizophrenia, bipolar disorders, generalized tissue sclerosis and many vision impairing and blinding diseases such as cataracts, glaucoma and retinopathies etc. While all these diseases inflict significant functional disabilities, the vision impairment due to cataract has one of the most adverse impacts on life, functionally as well as economically. Its prevalence is also very high. Everyone encounters it soon after the 40's when deterioration in vision perception due to lens changes becomes functionally noticeable and continues to decline thereafter. As summarized in Figure 1 A, cataracts account for about 49% of vision impairment in the world (1). Seemingly, the occurrence of the disease is somewhat higher in women than in men. Hence, one of the major thrust of eye research, at least till recently, has been directed towards understanding the pathogenesis of the disease and its possible therapeutic prevention. The thrust of such research however appears to have been decreased somewhat due to the advancements in surgical techniques of removing the cataractous lenses and its replacement by a synthetic implant. While this leads to an immediate satisfactory recovery of vision, the overall prevalence of the disease continues to remain high and essentially unchanged due to the inevitable replacement of the old cases by new ones. Therefore, surgery alone is unable to cope up with the problem. In addition, like with any surgery, there is always the possibility of inherent post-surgical complications which in this case can consist of a posterior capsular tear and consequent penetration of vitreous in the anterior chamber, cystoid macular edema, and often noted detachment of vitreous from the retina with photopsia and appearance of floaters (2, 3). The eye also has been reported now to be more prone to retinal detachment with significant loss of vision (4, 5). Removal of cataract has also been understood to hasten the appearance of macular changes associated with aging and diabetes. Hence it would be desirable to develop therapeutic measures to prevent or delay the process of cataract formation. Several experimental (6–8) and epidemiological studies (9, 10) strongly suggest the possibility that this can be achieved by use of antioxidants and certain metabolic agonists.

Implications of UV irradiation in Cataract formation

It has been known for a long time that the incidence of cataract is high in countries with excessive sun light. This has now been well established by compilation of the data by WHO (1), reproduced in Table 1 and figure 1A. As would be further obvious by referring to Figures 1B and 1C, most of the countries with high incidence of cataracts are located in areas of the world with relatively higher UV index (erythemal index) shifting annually between 0 to 35 degree north and south. Cataracts extracted from such countries are also known to be more strongly pigmented with yellow to brown coloration, than those extracted from patients native to the countries in higher latitudes (11–13). It has been suggested that such coloration as well as its higher incidence in countries with higher solar light intensities are due to the penetration of UV in the eye and consequent photo oxidation of proteins (11–13). This has been particularly ascribed to photo oxidation of tryptophan moieties. However, the absorption maxima of tryptophan and other aromatic amino acids present free as well as

part of the lens proteins is around 280 nm, wavelengths which do not penetrate the eye. Light only of wavelengths greater than 300 nm appear to do so. Therefore the correlation between the incidence data and the solar irradiation zones should be related to the pathophysiological effect of light primarily of wavelengths greater than 300 nm and beyond-although the contribution of visible light has received less attention. Many previous studies have suggested that the effect of UV on cataract formation involves photo-sensitization by tryptophan derived products generated metabolically, chiefly the kynurenine derivatives (11, 14). The absorption maxima of free kynurenine and hydroxyl kynurenine are near 368 nm (15), with a molar extinction coefficient of $3650 \text{ M}^{-1} \text{ Cm}^{-1}$. This is subject to minor shifts when present in combination with proteins, and with changes in pH. It increases to $4300 \text{ M}^{-1} \text{ Cm}^{-1}$ when metabolically converted to 3-hydroxy glucoside.

Based on the observation that exposure of animals (16) as well as humans to high oxygen atmosphere (17) and the relatively wide prevalence of cataract in areas of the world with excessive sunlight, we hypothesized that the high incidence of cataracts in countries with excessive light could be due to photochemical generation of reactive oxygen species (ROS) including superoxide and its derivatization to other potent entities such as hydrogen peroxide, hydroxyl radicals and singlet oxygen, in the aqueous and the lens and consequent oxidative damage (18–20). This can happen even by photosensitization in blue light (20)-light and oxygen acting synergistically in the presence of an appropriate photosensitizer. One of the earliest compounds known to be biologically strongly active photodynamically is acridine (21), pharmacologically used as acriflavine for external wound healing. Hence in initial examination of the hypothesis proposing that a continued photochemical generation of reactive oxygen might indeed be involved in causing oxidative stress to the lens we used riboflavin, known to be present as such as well as FAD and FMN in cornea (22), aqueous humor (23) and lens (24). Because of its high absorption maxima at 360, 450, 460 and 475 nm with extinction coefficients varying between $12,500$ and $11,000 \text{ M}^{-1} \text{ Cm}^{-1}$ (25), values which are about 3 times higher than that of kynurenine, we thought it would photosensitize the lens more strongly and would do so in near UV as well as under visible light. In addition, we thought that it should be capable of acting as a pseudocatalyst, converting molecular oxygen to its more reactive forms cyclically and thus be able to perpetuate the generation of ROS, in visible as well as in near UV, by integration of the following reactions. As shown below, the system will continue to do so as long as light enters the eye, and oxygen and reducing equivalents remain available to consistently reduce activated riboflavin molecules. Only a trace amount of this or other photosensitizer would be required.

Photochemical Generation of Reactive Oxygen Species

We first tested the effectiveness of the above photochemical system in causing lens damage in the rat lens organ culture system, measuring the transport of rubidium ions against its electro chemical gradient in the presence and absence of various scavengers of ROS and noticing the status of its transparency (19, 20). As shown in figure 2, when lenses were cultured in medium 199 containing $50 \mu\text{M}$ riboflavin phosphate for an overnight period of 18 hours in dark, the concentration of the rubidium ions in the lens rose to about 22 fold higher than in the medium-showing its accumulation in the lens against a concentration gradient. This value was similar to the value obtained with lenses incubated in the basal medium with riboflavin but in dark. Incubation in light using a day light tube adjusted to an

intensity of 50 ft. candle was found highly injurious to the tissue, the distribution ratio of rubidium ions in this case being reduced to 5. That this was related to the generation of ROS such as superoxide and hydrogen peroxide was evident by the preventive effect of ferricytochrome c as well as catalase. Significance of superoxide and hydrogen peroxide generation in the photo damage to the lens was further obvious by using the above system of incubation with potassium ferricyanide and potassium ferrocyanide (26). Addition of the ferricyanide to the medium which scavenges superoxide was nearly fully protective. Addition of ferrocyanide was also protective (Table 2). This is explained by the re-oxidation of the ferrocyanide to ferricyanide by the hydrogen peroxide produced simultaneously by the dismutation of superoxide going on in the same medium. Thus, the two agents work in concert, one scavenging superoxide and the other scavenging hydrogen peroxide. The lenses also remained very clear.

The importance of the superoxide generation in the oxidative cascade leading to lens opacification and cataract formation has now been further substantiated by studies with SOD knock out mice showing that the knockouts develop spontaneous cataracts (27, 28). However despite the presence of these enzymes human cataract development with aging and certain diseases such as diabetes still is a fact suggesting that the effectiveness of SOD and other antioxidant enzymes could be limited due to several reasons such as their deactivation with aging and their selective distribution and availability in various cellular compartments. Also, being macromolecules, they cannot penetrate to certain sensitive sites of oxidation buried in proteins and in nucleic acids. In addition, the lens is surrounded and bathed by aqueous and the vitreous humors, fluids that lack the enzymatic defenses. Therefore the lens cell membranes which remain continuously exposed to a photochemical oxidative environment due to the continued light penetration during the long periods of photopic vision remain susceptible to photo damage. Investigations were hence conducted to find out additional modes involved in protecting lens against oxidative stress.

We have hypothesized that is offered by ascorbate as one of the protective compounds (20). The concentration of this substance is about 20 fold higher in the aqueous than in the blood. Consequently, it is high in the lens also. Such a high concentration is maintained by its active transport from the plasma across the blood aqueous barrier. As shown in Table 3 its concentration in the aqueous and the lens is significantly higher in comparison to many other tissues and fluids (29). Additionally, as summarized in Table 4 its level in the diurnal animals is significantly higher than that in the nocturnal animals, pointing out to a natural biological adaptation in response to the photochemical environment in the aqueous and lens of the diurnal animals (29). The initial verification of the ascorbate hypothesis was done by lens culture experiments directly in light in the riboflavin containing medium (20). As shown in figure 3 addition of ascorbate to the medium was significantly effective in preventing photochemical damage to lens transport pump. The results on experiments with peroxide generated by the xanthine-xanthine oxidase system in dark are summarized in Table 5 (30). As summarized in Table 6, the rate constant of its reaction with the hydroxyl radical is significantly high and hence is controlled essentially only by its diffusion (31). However, while ascorbate could be directly effective in protecting the tissue by scavenging ROS in the aqueous milieu, its effectiveness in the lipid environ of the tissue could become partial by limiting its availability primarily to the aqueous region. Vitamin E, which is lipid soluble, could therefore be the compound of choice to protect the tissue lipids. This is indicated by preventive effect of this nutrient when fed to Emory mice, diabetic rats and UV-exposed rabbits (32, 33, 6)

However, the availability of such nutritional antioxidants can be varied, depending upon the agricultural seasons, dietary variations and many physiological factors such as the effectiveness of their gastrointestinal absorption. The efficiency of their transport through

the blood aqueous barrier can also decline with age. Interestingly, its concentration in the aqueous humor of cataractous patients have been reported to be lower than normal (34). In addition, there seems to be some correlation with the type of cataract and metabolic status of the patients such as status of tissue glycolysis.

We therefore hypothesized that pyruvate, produced normally by tissue metabolism can also participate in the delay of cataracts. It is well known to react with various ROS generating acetate and carbon dioxide (35–39). The rate constants of these reactions as summarized below are also sufficiently high.

Reactivity of Pyruvate with Reactive Oxygen Species

	ΔG°	K, Cal/mole	K'eq
$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$	-29.30		3.06×10^{21}
$Pyruvate + H_2O_2 \longrightarrow Acetate + CO_2 + H_2O$	-77.95		1.46×10^{21}
$2O_2^- + 2H^+ + Pyruvate \longrightarrow Acetate + CO_2 + H_2O + O_2$	-107.25		4.5×10^{23}
$Pyruvate + OH + Fe^{2+} \longrightarrow Acetate + CO_2 + Fe^{3+}$			

Rate constant: $5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ to $9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$

As shown in figure 4, its incorporation in the medium of incubation was found highly effective against oxidative modification of the proteins inflicted by incubation of the tissue in presence of hydrogen peroxide (40). It was also found effective in preventing oxidative damage to the rat lens in terms of the decreased ability to carry out active transport of rubidium ions when incubated with xanthine and xanthine oxidase (41) or in riboflavin containing medium exposed to day light (42). Similar effects have been observed also in the case of mouse lens (43) where the incubation medium contained xanthine oxidase as well as uricase (Figure 5). More recently, we have demonstrated that its protective effect, in addition to being exerted by its ROS scavenging activity can also be attributed to its ability to stimulate the payoff portion of tissue glycolysis (44), and thereby help in the maintenance of tissue levels of ATP, required for supporting the biosynthetic activities in addition to active transport. In all its reactions with various ROS, the products generated are acetate, carbon dioxide and water. Hence no toxic products are generated, unlike the case with ascorbate and vitamin E where products of reaction containing a keto group (dehydroascorbate and tocopherones) can potentially act as protein cross-linking agents. They can also become pro-oxidants in presence of certain metal ions. Pyruvate on the other hand, in addition to its radical scavenging properties, also inhibits competitively the process of protein glycation by sugars, an important reaction involved with aging and cataract formation, particularly in diabetes (45). Encouraged with these beneficial properties, experiments have also been done to examine its effectiveness in preventing cataract formation in vivo using galactosemic and diabetic animal models. Maintaining rats as well mice on water containing 2% sodium pyruvate has been found to be significantly effective in preventing cataract formation (7, 46–48). As shown in Figures 6 & 7, lenses in both the species maintained on pyruvate-containing water and food were much more transparent than in the group receiving regular diet and water.

In addition to its effectiveness against diabetic cataract formation, we also demonstrated that it is effective in preventing oxyradical induced lens damage triggered by UVA irradiation (49). The inhibition of rubidium uptake induced by ROS was significantly prevented by pyruvate. The biochemical damage reflected by the decrease in the levels of GSH was also substantially minimized (Figure 8). The frank appearance of cataracts (Figure 9) as well as the associated histological changes (Figures 10, 11) was also prevented.

Therefore, these and several other studies with antioxidants strongly suggest that cataract is a pharmacologically preventable disease. However, it would be necessary to carry on such treatment only topically and not systemically. This is because of the greater effectiveness of compounds when administered topically. In addition, it is desirable to use this route to avoid

toxicity of the compounds which will then be required to be given in larger amounts if one uses the oral route. However achieving this topically is problematic because of the instability of most of the antioxidants when compounded pharmaceutically. In addition, in the eye they get photolyzed due to continued light penetration, especially during the long period of photopic vision. It was therefore decided to investigate cataract prevention by antioxidants and free radical scavenging compounds that are relatively more stable and well tolerated physiologically by the human body.

In this connection, we have recently observed that caffeine which is much more stable than ascorbate, vitamin E and pyruvate is highly effective in protecting the lens against UV-induced damage. This was initially ascertained by incubation lens studies under UVB (300nm) conducted in basal Tyrode medium pulsed with $^{86}\text{Rb}^+$ without or with caffeine (50). Subsequent to incubation, they were examined for the state of their transparency, extent of $^{86}\text{Rb}^+$ accumulation and the contents of ATP and GSH. As expected, incubation under UV was associated with the loss of transparency as monitored photographically (Figure 12). $^{86}\text{Rb}^+$ accumulation was also inhibited, with associated loss of ATP and GSH. In the presence of caffeine, the lenses remained much more transparent. The extent of $^{86}\text{Rb}^+$ accumulation as well as the contents of GSH and ATP was also significantly higher (Table 7). Further confirmation of the effectiveness of caffeine against oxyradical species was also done in dark where ROS was generated by Fe_8Br_8 a water soluble iron complex (51). It exerted similar inhibitory effects on the rubidium accumulation and loss of transparency. The contents of GSH and ATP were also decreased (Table 8). In order to confirm further that the effect of caffeine observed above is due to its ability to scavenge ROS, parallel studies were conducted with Tempol, a more conventional oxyradical scavenger which also was found effective (Table 9). However, since UV wavelength used in the above experiments were lower than the UV that normally enters the eye, further confirmation of the protective effect of caffeine against UV damage was obtained by lens culture experiments at 365 nm, maintaining similar irradiation intensity of 0.6 mW/sq. cm (52). As expected, the effect of such irradiation does not significantly affected -the rubidium pump damage. However addition of kynurenine to the medium significantly enhanced the damaging effect of UVA, the uptake of rubidium in this case being only 20% of the basal controls. Addition of caffeine was again highly protective, the uptake of rubidium in this case being 85% of the basal dark control. It also maintained the levels of ATP and GSH closer to the control (Table 10). Figure 13 represents the ESR spectroscopy of the medium exposed to UVA. As apparent, hydroxyl quartet signals were well noticeable. The signals were minimized significantly by addition of caffeine. Thus one of the primary modes of the caffeine effect appears to be related to its ability to scavenge the ROS produced in the medium by photo-irradiation. Hence studies are in progress to test its effectiveness in vivo. At present we are limited by the lack of an appropriate animal model of UV cataracts. However, since oxidative stress is an important factor in the genesis of cataracts in general including UV irradiation, we have examined the effectiveness of caffeine against cataract formation in selenite and galactosemic animal models (53–55). As will be obvious by reference to figure 14, administration of caffeine to sodium selenite treated rats was highly preventive, indicated by the inhibition of cataract formation as well as by the maintenance of the levels of ATP and GSH. Similar results were obtained with galactosemic rats where they were given caffeine mixed with the diet (54). However in view of the desirability of treating eye diseases with topical eye drops, further experiments with galactosemic animals have been done with caffeine eye drops (55), such mode of treatment being expected to be more efficacious while at the same time avoiding administration of the compound orally in larger amounts. Hence, in these experiments, the galactosemic animals were treated with eye drops containing about 280 μg of caffeine, 5 times/day and cataract formation was followed for 25 days. As indicated in Figure 15, the progress of cataract was significantly thwarted by caffeine treatment. The extent of opacity till the end of the experiment was only minor if any

in the caffeine eye drops treated group. In the untreated (placebo) group cataract was highly advanced occupying the entire lens. The prevention of cataract was also evident by higher levels of GSH (Figure 16). The protective effect was further apparent by H&E histology (Figure 17). In the caffeine untreated group, the sub-epithelial and deeper cortical layers of the tissue had abundance of nucleated fiber cells representing aberrantly differentiating cells. The cells are also hydropic with the overall structure becoming non-homogeneous. On the contrary, lenses of the animals treated with caffeine eye drops maintained a normal structure consisting of a single layer of epithelial cells followed by well-aligned non-nucleated fiber cells. The central region of the tissue also remained homogenous. As we have previously established, the cataractogenic process eventually leads to apoptotic cell death. That was also found to be inhibited by caffeine. This was done by staining the lens sections with DAPI and F-dUTP (Figure 18). As expected, most of the nucleated cells are in the bow zone and the anterior epithelium in the normal tissue. In the case of galactose-fed animals, the distribution of DAPI-positive cells was highly abnormal, showing abundance of nucleated cells in the deeper cortex. That these cells are indeed apoptotic was proved by TUNEL staining using F-dUTP. In the galactosemic animals treated with caffeine, the apoptotic cells were essentially absent and the distribution of the cells was also normal as expected.

The anti-apoptotic effect of caffeine is also now being studied in lenses exposed directly to UV, preliminary results being similar to that in galactosemic animals. In conclusion, therefore, it has been shown that oxyradical generation is an important pathogenic factor in the genesis of cataracts induced concomitant to the penetration of UV as well as visible light. We have demonstrated that such cataract formation can potentially be prevented by treatment with compounds which can act as oxyradical scavengers when given in small amounts through topical eye drops. In view of the instability of ascorbate and vitamin E when incorporated into pharmaceutical preparations as well as in the eye due to photolytic decomposition, it is apparent that some more stable compounds such as the pyruvate esters and xanthine alkaloids such as caffeine might be found useful.

Acknowledgments

The work was supported by NEI, NIH through an RO1 grant EY01292 to SDV.

Supported by NIH NEI grant RO1 EY01292 to S. D. Varma

References

1. Resnikoff S, Pascolini D, Etya'ale D, et al. Global data on visual impairment in the year 2002. Bulletin WHO. 2004; 82:844–851.
2. Stark WJ Jr, Maumenee AE, Fagadau W, Datiles M, Baker CC, Worthen D, Klein P, Auer C. Cystoid macular edema in pseudophakia. Surv Ophthalmol. 1984; 28(suppl):442, 451. 1984. [PubMed: 6463847]
3. Fine BS, Brucker AJ. Macular edema and Cystoid macular edema. Am J Ophthalmol. 1981; 92:466–481. [PubMed: 7294109]
4. Clayman HM, Jaffe NS, Light DS, Jaffe MS, Cassady JC. Intraocular lenses, axial length, and retinal detachment. Am J Ophthalmol. 1981; 92:778–780. [PubMed: 7315928]
5. Smith PW, Stark WJ, Maumenee AE, Enger CL, Michels RG, Glaser BM, Bonham RD. Retinal detachment after cataract extraction with posterior chamber intraocular lens. Ophthalmology. 1987; 94:495–504. [PubMed: 3601365]
6. Robertson JM, Donner AP, Trevithick JR. A possible role for vitamins C and E in cataract prevention. Am J Clin Nutr. 1991; 53(1 Suppl):346S–351S. [PubMed: 1985408]
7. Zhao W, Devamanoharan PS, Henein M, Ali AH, Varma SD. Diabetes-induced biochemical changes in rat lens: attenuation of cataractogenesis by pyruvate. Diabetes Obes Metab. 2000; 2(3): 165–74. [PubMed: 11220552]

8. Hegde KR, Varma SD. Prevention of cataract by pyruvate in experimentally diabetic mice. *Mol Cell Biochem.* 2005; 269(1–2):115–120. [PubMed: 15786723]
9. Jacques PF, Hartz SC, Chylack LT Jr. McGandy RB, Sadowski JA. Nutritional status in persons with and without senile cataracts: blood vitamin and mineral levels. *Am J Clin Nutr.* 1988; 48:152–158. [PubMed: 3389322]
10. Taylor A, Jacques PF, Chylack LY Jr. et al. Long-term intake of vitamins and carotenoids and odds of early age-related cortical and posterior subcapsular lens opacities. *Am J Clin Nutr.* 2002; 75:540–549. [PubMed: 11864861]
11. Pirie A. Formation of N'-Formylkynurenine in proteins from lens and other sources by exposure to sunlight. *Biochem J.* 1971; 125:203–208. [PubMed: 5168391]
12. Lerman S, Borkman R. Spectroscopic evaluation and classification of the normal aging and cataractous lens. *Ophthalmol Res.* 1976; 8:335–353.
13. Grover D, Zigman S. Coloration of human lens by near UV photolyzed tryptophane. *Exp Eye Res.* 1972; 23:70–75. [PubMed: 5060115]
14. Van Henyningen R. Fluorescent glucosides in the human lens. *Nature.* 1970; 230:393–394.
15. Bando M, Nakajima A, Satoh K. Spectrophotometric estimation of 3-OH L-Kynurenine-O-Beta-Glucoside in human lens. *J Biochem.* 1981; 89:103–109. [PubMed: 6783628]
16. Schocket SS, Esterson J, Bradford B, et al. Induction of cataracts in mice by exposure to oxygen. *Israel J Med Sci.* 1972; 8:1596–1601. [PubMed: 4647826]
17. Palmquist B-M, Philipson B, Barr P-O. Nuclear cataract and myopia during hyperbaric oxygen therapy. *Br J Ophthalmol.* 1984; 68:113–117. [PubMed: 6691953]
18. Varma SD, Ets TK, Richards RD. Protection against superoxide radicals in rat lens. *Ophthalmic Res.* 1977; 9:421–431.
19. Varma SD, Chand D, Sharma YR, Kuck JF Jr. Richards RD. Oxidative stress on lens and cataract formation. Role of light and oxygen. *Curr Eye Res.* 1984; 3:35–57. [PubMed: 6360540]
20. Varma SD, Kumar S, Richards RD. Light-induced damage to ocular lens cation pump: prevention by vitamin C. *Proc Natl Acad Sci U S A.* 1979; 76(7):3504–3506. [PubMed: 291017]
21. Blum HF. Photodynamic action. *Physiol Rev.* 1932; 12:23–55.
22. Bessey OA, Lowry OH. Factors affecting the riboflavin content of the cornea. *J Biol Chem.* 1944; 155:635–643.
23. Philpot PJ, Pirie A. Riboflavin adenine dinucleotide in ox ocular tissue. *Biochem J.* 1943; 37:250–254. [PubMed: 16747626]
24. Kinsey EV, Frohman CE. Studies on crystalline lens. Distribution of cytochrome, total riboflavin, lactate and pyruvate and their metabolic significance. *AMA Archives of Ophthalmology.*
25. Macheroux, P. UV-visible spectroscopy as a tool to study flavoproteins. In: Chapman, SK.; Reid, GA., editors. *Methods Mol Biol* (Vol 131) Flavoprotein protocols. Humana Press Inc.; Totowa, NJ: 1999. p. 1-2.
26. Varma SD, Mooney JM. Photodamage to the lens in vitro: implications of the Haber-Weiss reaction. *J Free Radic Biol Med.* 1986; 2:57–62. [PubMed: 2430008]
27. Behndig A, Karlsson K, Reaume AG, Sentman ML, Marklund SL. In vitro photochemical cataract in mice lacking copper-zinc superoxide dismutase. *Free Radic Biol Med.* 2001; 31:738–744. [PubMed: 11557311]
28. Reddy VN, Kasahara E, Hiraoka M, Lin LR, Ho YS. Effects of variation in superoxide dismutases (SOD) on oxidative stress and apoptosis in lens epithelium. *Exp Eye Res.* 2004; 79:859–868. [PubMed: 15642323]
29. Varma SD, Richards RD. Ascorbic acid and the eye lens. *Ophthalmic Res.* 1988; 20(3):164–73. [PubMed: 3186190]
30. Varma SD, Morris SM, Bauer SA, Koppenol WH. In vitro damage to rat lens by xanthine-xanthine oxidase: protection by ascorbate. *Exp Eye Res.* 1986; 43(6):1067–1076. [PubMed: 3817025]
31. Cabelli DE, Bielski JH. Kinetics and mechanism of oxidation of ascorbate by H₂O₂/O₂ radicals. A pulse radioisotope and stopped flow study. *J Phys Chem.* 1983; 87:1809–1832.
32. Varma SD, Devamanoharan PS, Mansour S, Teter B. Studies on Emory mouse cataracts: oxidative factors. *Ophthalmic Res.* 1994; 26(3):141–8. [PubMed: 8090431]

33. Ayala MN, Soderberg PG. Vitamin E can protect against ultraviolet radiation-induced cataract in albino rats. *Ophthalmic Res.* 2004; 36(5):264–9. [PubMed: 15583432]
34. Chandra DB, Varma R, Ahamad S, Varma SD. Vitamin C in the human aqueous humor and cataracts. *International Journal of Vitamin and Nutrition Research.* 1986; 56:165–168.
35. Fenton HJH, Jones HO. The Oxidation of organic acids in presence of ferrous iron, Part I. *J Chem Soc Trans.* 1900; 77:69–76.
36. Holleman MAF. Note on the action of oxygenated water on alpha-keto acids and 1,2, dicetones. *Recl Tran Chi Pays-bas Belq.* 1904; 23:169–172.
37. Ervens B, Gligorovski S, Herrmann H. Temperature dependent rate constants for hydroxyl radical reactions with organic compounds in aqueous solutions. *Phys Chem Chem Phys.* 2003; 5:1811–1824.
38. Millouki A, Mu YJ. On atmospheric degradation of pyruvic acid in the gas phase. *J Photochem Photobiol A Chem.* 2003; 157:295–300.
39. Kraljic, I. Chemistry of ionization and excitation. Johnson, GRA.; Scholes, G., editors. Taylor and Francis Ltd.; London: 1967. p. 303-309.
40. Varma SD, Ramachandran S, Devamanoharan PS, et al. Prevention of oxidative damage to rat lens by pyruvate in vitro: possible attenuation in vivo. *Curr Eye Res.* 1995; 14:643–649. [PubMed: 8529399]
41. Varma SD, Morris SM. Peroxide damage to the eye lens in vitro. Prevention by pyruvate. *Free Radical Research Communications.* 1988; 4:283–290. [PubMed: 3234857]
42. Varma SD, Devamanoharan PS, Morris SM. Photoinduction of cataracts in rat lens in vitro. Preventive effect of pyruvate. *Exp Eye Res.* 1990; 50:805–812. [PubMed: 2373172]
43. Varma SD, Hegde K, Henein M. Oxidative Damage to mouse lens in culture. Protective effect of pyruvate. *Biochim Biophys Acta.* 2003; 1621:246–252.
44. Hegde KR, Kovtun S, Varma SD. Inhibition of glycolysis in retina by oxidative stress. Prevention by pyruvate. *Mol Cell Biochem.* 2010; 343:101–105. [PubMed: 20559692]
45. Devamanoharan PS, Ali AH, Varma SD. Non-enzymatic glycation of lens proteins and hemoglobin. Inhibition by pyruvate. An in vivo study. *Diabetes, Obesity and Metabolism.* 1999; 1:159–164.
46. Kalakonda S, Hegde KR, Varma SD. Ophthalmoscopic and morphogenetic changes in rat lens induced by galactose: attenuation by pyruvate. *Diabetes Obesity Metabolism.* 2004; 6:216–22.
47. Varma SD, Hegde KR, Kovtun S. Attenuation and delay of diabetic cataracts by antioxidants: Effectiveness of pyruvate after onset of cataract. *Ophthalmologica.* 2005; 219:309–315. [PubMed: 16123559]
48. Hegde KR, Varma SD. Morphogenetic and Apoptotic Changes in Diabetic Cataract. Prevention by Pyruvate. *Mol Cell Biochem.* 2004; 262:233–237. [PubMed: 15532728]
49. Hegde KR, Kovtun S, Varma SD. Induction of UV cataract in vitro. Prevention by pyruvate. *J Ocul Pharmacol Ther.* 2007; 23:492–502. [PubMed: 17900232]
50. Varma SD, Hegde KR, Kovtun S. UV-B induced damage to the lens in vitro. Prevention by caffeine. *Journal of Ocular Pharmacol & Therap.* 2008; 24:439–44.
51. Varma SD, Hegde KR. Prevention of oxidative damage to lens by caffeine. *J Ocular Pharmacol Therap.* 26:73–77. 210.
52. Varma SD, Hegde KR. Kynurenine induced photo oxidative damage to lens in vitro. Protective effect of caffeine. *Mol Cell Biochem.* 340:49–54. [PubMed: 20204679]
53. Varma SD, Hegde KR, Kovtun S. Inhibition of selenite-induced cataract by caffeine. *Acta Ophthalmol.* 2010; 88:e245–9. [PubMed: 20977689]
54. Varma SD, Hegde KR, Kovtun S. Oxidative stress in lens in vivo. Inhibitory effect of caffeine. A preliminary report. *Mol Vis.* 2010; 23:501–505. [PubMed: 20352023]
55. Varma SD, Hegde KR, Kovtun S. Effectiveness of topical caffeine in cataract prevention. Studies with galactose cataract. *Mol Vis.* In press.

Figure 1.

1A. Distribution of ocular diseases causing visual impairment (1).

1B & 1C: Global level of UV index (Erythemal index) at noon with seasonal shifts.

http://www.temis.nl/uvradiation/archives/uvief/2008/06/uvief20080624_w.gif

Figure 2.

Effect of riboflavin phosphate and light on the uptake of $^{86}\text{Rb}^+$. Effect of catalase, SOD, ferricytochrome C and ascorbic acid: A, Control lenses incubated in dark with riboflavin phosphate (50 μM). B, Lenses incubated as in A but in presence of light. C, Lenses incubated as in B but containing 10^{-6} M Ferricytochrome C. D, Lenses incubated as in B but containing 5×10^{-6} M ferricytochrome C. E, Lenses incubated as in B but containing catalase (15 Units/4ml). F, Lenses incubated as in B but containing 2.5mM L-ascorbic acid. The CL/CM remained similar if the incubation was conducted in light in medium without riboflavin phosphate (19).

Figure 3.

Protective effect of ascorbate against damage to the lens cation pump in the presence of light + Riboflavin: Rat lenses were incubated for 21 hrs as described under the legend of figure 2 and the distribution of $^{86}\text{Rb}^+$ between the in the lens and medium determined. Bars: A, control with or without riboflavin in the dark and without riboflavin in the light; B, with riboflavin in the light; C, with riboflavin and ascorbate in the light (20).

Figure 4.

Protective effect of pyruvate against oxidative damage to lens proteins incubated with hydrogen peroxide. This was ascertained by SDS-PAGE profile of urea soluble proteins isolated from the post incubation lenses. Molecular weight standards: 1: 14Kd, 2: 18Kd, 3: 29Kd, 4: 48Kd, 5: 56 Kd. *Lane #s starting from the left: Lane 1:* Freshly dissected lens; *Lane 2:* lens incubated in basal medium; *Lane 3:* Lens incubated with hydrogen peroxide (2mM); *Lane 4:* Lens incubated with 2mM hydrogen peroxide + 10mM sodium pyruvate. Majority of the high molecular weight proteins generated by oxidative stress are retained in the stacking gel in lane 3 (40).

Figure 5. Protective effect of pyruvate against damage to mouse lens cation pump caused by incubation with XA, XO and uricase. Red and yellow curves represent the uptake by lens incubated without and with pyruvate. Protective effect was measured in terms of the distribution ratio of rubidium (CL/CM) attained as a function of time (43).

Figure 6.

Prevention of galactose cataract by pyruvate. Scheimpflug slit-lamp pictures of the eyes of normal, galactose and galactose plus pyruvate fed rats: As expected, lenses in the normal group are optically quiescent, except after 18 days when some nuclear demarcation was perceptible. In the galactose-fed group, definitive early opacity could be noted by 7 days, followed by more advanced opacity on day 18. In the galactose-plus-pyruvate group, no lens opacity was apparent until 7 days. However, some opacity did appear by day 18. This was, however, substantially lower than that in the animals fed galactose without pyruvate (46).

Figure 7.

7A: Photographs showing inhibition of diabetic cataract formation in rat diabetic models. N = clear lenses in the eyes of basal controls, D = diabetic eye showing opacity, D+P = diabetic eye of animals treated with pyruvate. The central circle represents normal camera reflection. The opacity is marked by excessive light scattering appearing white in D. Pictures were taken after 80 days of streptozotocin injection (7). Pyruvate was given in drinking water as a 2% solution.

7B: Representative pictures of the mouse eyes taken with SL-45 Topcon slit lamp camera after mydriasis with 1% Tropicamide eye drops. N: Normal, D: Diabetic without treatment with pyruvate, C: Diabetic treated with pyruvate. The opacities in the untreated diabetic group are located centrally as well as posteriorly. In the pyruvate treated group, the lens appears close to the normal. Pyruvate was given to the diabetic group as 1% drinking water starting three weeks after the establishment of early cataracts (47).

Figure 8.

Prevention of UVA (365 nm)-induced damage to the lens cation pump in vitro by pyruvate. Freshly isolated rat lenses were incubated with 12.5 μ M riboflavin in the absence and presence of UV for 18 hours and then photographed after placing them on grid. Controls were run in dark. Level of pyruvate when used was 5mM.

Figure 9.

Prevention of UVA induced morphological damage to the lens by pyruvate. As apparent by light microscopy of the H & E stained sections of the entire lens (10 $\times$), the tissue structure has become heterogeneous and is characterized by the separation of epithelium and cortex and liquefaction in the nucleus on incubation with Riboflavin + UVA. In the presence of pyruvate, these changes were minimal (49).

Figure 10.

Examination of the equatorial region of the above lenses at 40 $\times$. The extensive separation of the fiber cells and their swelling is now highly evident in the lenses incubated without pyruvate, contrary to that in the presence of pyruvate where the fiber cells are well-aligned (49).

Figure 11.

Prevention of UVA-induced oxidative loss of GSH by pyruvate: Rat lenses were incubated exposed to UVA in medium 199 with and without 12.5 μ M riboflavin in the absence and presence of pyruvate. Dark controls were run simultaneously. GSH was determined in the 5% TCA extract of the lens as described previously (49).

Figure 12.

Development of lens opacity under UV-B. Prevention by caffeine (50): Mice lenses were incubated for 5 hours in Tyrode medium exposed to UV-B (302nm) in the absence and presence of caffeine (5mM). Subsequently, the lenses were placed on a grid and photographed by trans-illumination with white light. Transparency is indicated by the clarity of the holes which were much more obscured in the lenses incubated under UV as compared to that in the lenses incubated with UV + caffeine.

Figure 13.

ESR tracings of the Tyrode medium containing kynurenine exposed to UVA in the absence and presence of indicated levels of caffeine. The abscissa represents the magnetic field (Gauss) and the ordinate represents relative signal intensity (Arbitrary units). (A) The signals of the DMPO-OH* radical generated by incubation in UV was significantly attenuated by caffeine as shown in (B) and (C). The effect was concentration dependent (52).

Figure 14.

Prevention of selenite cataract by caffeine: Pictures of representative lenses in each group were taken by transillumination to demonstrate their state of transparency. As apparent, the lenses of the animals given sodium selenite are severely cataractous (U), the opacity being more dense in the nuclear than in the surrounding areas. In the majority (64%) of the caffeine treated pups (T_1), the lenses remained largely transparent. T_2 represents 36% of the caffeine treated animals where cataract prevention remained incomplete in comparison to T_1 , but the severity of transparency loss was still significantly less than that in group U (53).

Figure 15.

Prevention of galactose cataract by topical application of caffeine in an eye drop preparation. The animals were euthanized at indicated time periods, lenses dissected out and placed on a Millipore metallic grid with uniform holes. Photographs were then taken in dark with retro-illumination. Cataract is indicated by light obstruction through the holes and its scattering. Noticeable is the presence of advanced cataract on day 25 in the caffeine untreated group and the absence of such advanced opacity in the caffeine-treated group (55).

Figure 16.

Loss of glutathione in galactosemic lens. Prevention by topical caffeine administration. The experimental protocols were similar to that described under the legend of figure 14. The level of GSH in the galactose plus caffeine is always significantly higher than that in the galactose alone group. The levels in the caffeine group are closure to the basal controls (55)

Figure 17.

H&E profile of the lens sections. The upper panel represents lenses in rats given the galactose diet treated only with the placebo. Apparent is the retention of nucleus in the fiber cells due to defective cellular transition. The nuclei are also clearly pyknotic. The central area of the tissue also contains some nucleated cells. Other cells are swollen and detached from each other. These abnormalities were inhibited in the group treated with caffeine eye drops as shown in the lower panel (55).

Figure 18.

Inhibition of galactose-induced apoptosis by caffeine eye drops: The pictures represent the sections of the lens stained with DAPI (1–4) and F-UTP (5–8). Sections labeled 1 and 5 represent normal lenses. # 2 and 6 represent the lenses from normal rats treated with caffeine eye drops. Sections 3 and 7 represent lenses from galactose group treated with placebo. Sections 4 and 8 represent caffeine treated group. Reference to # 3 and 7 indicate defective transformation of the epithelial cells into fiber cells, indicated by retention of nuclei and their apoptosis. Sections 4 and 8 prove that caffeine inhibits such apoptotic process (55).

Table 1

Global incidence of eye diseases (1)

Causes of overall visual impairment in world population due to various eye diseases in terms of prevalence in WHO sub regions as of 2002. Afr, WHO African region; Amr, WHO Region of the Americas; Emr, WHO Eastern Mediterranean Region; Eur, WHO European Region; Sear, WHO South-East Asia Region; Wpr, WHO Western Pacific Region.

Region	Cataract	Glaucoma	AMD ^a	Corneal opacities	Diabetic retinopathy	Childhood blindness	Trachoma	Oncho-cerciasis	Others
Afr-D	50	15		8		5.2	6.2	6	9.6
Afr-E	55	15		12		5.5	7.4	2	3.2
Amr-A	5	18	50	3	17	3.1			3.9
Amr-B	40	15	5	5	7	6.4	0.8		20.8
Amr-D	58.5	8	4	3	7	5.3	0.5		13.7
Emr-B	49	10	3	5.5	3	4.1	3.2		22.2
Emr-D	49	11	2	5	3	3.2	5.5		213
EUr-A	5	18	50	3	17	2.4			4.6
Eur-B1	28.5	15	15	8	15	3.5			15.0
Eur-B2	35.5	16	15	5	15	6.9			6.6
Eur-C	24	20	15	5	15	2.4			18.6
Sear-B	58	14	3	5	3	2.6			14.4
Sear-D	51	9	5	3	3	4.8	1.7		22.5
Wpr-A	5	18	50	3	17	1.9	0.025		5.0
Wpr-B1	48.5	11	15	3	7	2.3	6.4		6.8
Wpr-B2	65	6	5	7	3	3.6	3.5		6.9
Wpr-B3	65	6	3	3	5	9.5	4.3		4.2
World	47.8	12.3	8.7	5.1	4.8	3.9	3.6	0.8	13.0

^a AMD, age-related macular degeneration (1)

Table 2
Uptake of RB-86 by the Rai Lens Incubated in Light in Medium Containing Riboflavin.
Effect of Various Scavengers

Photochemical damage to the rat lens rubidium pump activity in vitro. Protective effect of ROS scavengers. Incubations were one in medium 199 containing 50 μ M riboflavin and pulsed with rubidium-86 chloride. The blank controls consisted of lenses incubated in the dark. In other cases, the controls and corresponding experimentals were incubated exposed to fluorescent day light for a period of 18 to 20 hours and the distribution ratio of the rubidium ions between the lens water (CL) and the medium (CM) determined as described previously (26). The values have been expressed as mean $\pm$ standard deviation. An asterisk indicates that the values are significantly different from the contralateral controls; the p values being <0.001.

Conditions	n	$\bar{x}_{CL} / CM$	$\bar{x}_E / C \times 100$
Blank control	20	22.0 $\pm$ 3.0	
-SOD (control)	20	5.06 $\pm$ 2.27	
+ 8 units Mn SOD	9	9.19 $\pm$ 2.98	166.9 $\pm$ 26.9*
+ 20 units Mn SOD	11	10.04 $\pm$ 2.75	198.2 $\pm$ 34.8*
-Catalase (control)	20	6.5 $\pm$ 2.5	
+4 units catalase	10	20.0 $\pm$ 3.0	307.0 $\pm$ 80.0*
+16 units catalase	10	21.0 $\pm$ 2.0	323.0 $\pm$ 80.8*
-Na ₃ Fe(CN) ₆ (control)	16	7.44 $\pm$ 1.43	
+ 5 μ M Na ₃ Fe(CN) ₆	8	20.44 $\pm$ 2.35	213.2 $\pm$ 39.8*
+ 10 μ M Na ₃ Fe(CN) ₆	8	20.88 $\pm$ 3.97	280.2 $\pm$ 18.4*
-Na ₄ Fe(CN) ₆ (control)	10	7.00 $\pm$ 1.47	
+ 5 μ M Na ₄ Fe(CN) ₆	5	19.55 $\pm$ 2.00	
+ 10 μ M Na ₄ Fe(CN) ₆	5	19.96 $\pm$ 3.86	223.3 $\pm$ 15.1*
-mannitol (control)	7	6.02 $\pm$ 3.20	
+ 100 μ M mannitol	8	11.05 $\pm$ 3.00	138.9 $\pm$ 34.5*
+ FeSO ₄ (5 μ M) (control)	8	8.33 $\pm$ 2.19	
+ FeSO ₄ (5 μ M)			
+ 100 μ M mannitol	10	12.90 $\pm$ 3.07	156.6 $\pm$ 31.3*

Table 3
Ascorbate concentration in human and other mammalian tissues (mg/kg)

Data have been compiled from The Biochemists' Handbook, Cyril Long, Ed., Van Nostrand Publishers, Princeton, 1991

BRAIN	110	ADRENAL	220		
PANCREAS	95	CORNEA	240		
LIVER	64	LENS	250	SALIVA	1
SPLEEN	81	AQUEOUS HUMOR	200	SEMINAL VESICLE SECRETION	50
KIDNEY	47	VITREOUS	360	URINE	2-25
LUNG	45	RETINA (DOG)	115	BLOOD SWBC & PLATELETS	10 M
HEART	21	CHOROIDS (DOG)	250	SERUM	10 MG
THYMUS	45	CILIARY BODY (DOG)	90	CSF	10 MG
WHITE BLOOD CELLS & PLATELETS	250	SCLERA (RABBIT)	140		
EPIDIDYMIS	140				
OVARY (S CORPUS LUTEUM)	280	TOTAL BODY STORE ABOUT 4 GM.(54MG/KG)			

Table 4
Levels of Vitamin C in Lens and Aqueous Humor

Data have been compiled from The Biochemists' Handbook, Cyril Long, Ed., Van Nostrand Publishers, Princeton, 1991

Species	Aqueous humor, mM	Lens, millimoles/kg wet weight
1. Man	1.00 ± 0.20	1.25 ± 0.45
2. Monkey	1.00 ± 0.10	Unknown
3. Horse	1.20 ± 0.20	3.02 ± 0.20
4. Ox	1.08 ± 0.20	2.04 ± 0.45
5. Rabbit	1.70 ± 0.60	0.74 ± 0.34
6. Sheep	1.20 ± 0.20	1.81 ± 0.60
7. Guinea Pig	0.75 ± 0.50	0.65 ± 0.10
8. Pig	0.57 ± 0.03	1.20 ± 0.35
9. Dog	0.31 ± 0.03	0.20 ± 0.10
10. Cat	0.07 ± 0.04	--
11. Galago	0.03 ± 0.02	--
12. Rat	0	0.08 ± 0.08

Compiled from The Biochemists' Handbook, Cyril Long, Ed., Van Nostrand Publishers, Princeton, 1991

Table 5
Effect of ascorbic acid on rubidium and AIB uptake

The effectiveness of ascorbate in preventing oxidative damage was determined by incubation of lenses as described table 2, but in dark in terms of the distribution ratio of the rubidium (Rb-86) and alpha-aminoisobutyric acid (AIB). Lenses were incubated as control C and experimental (E) with additions as indicated in the table. (31).

Additions to the medium		CL/CM	E/C × 100	n
Rubidium uptake				
C	None	21 ± 1.5		(6)
E	Na ascorbate, 1.0 mM	21 ± 2.0	100	(2)
E	Na ascorbate, 2.0 mM	21.5 ± 0.5	102	(3)
C	XA	19 ± 1.5		
E	XA + XO, 0.1 unit	4.5 ± 1.0	24	(6)
C	XA + XO, 0.1 unit	5.2 ± 0.5		
E	XA + XO + Na ascorbate, 2 mM	10.4 ± 0.4	200	(10)
C	XA + XO, 0.10 unit	4.54 ± 0.4		
E	XA + XO, 0.1 unit + DHA, 2.5 mM	4.78 ± 0.28	105	(5)
AIB uptake				
	XA	5.5 ± 0.5	—	(4)
C	XA + XO, 0.1 unit	1.48 ± 0.38		
E	XA + XO, 0.1 unit + Na ascorbate, 2 mM	3.0 ± 0.47	203	(7)
C	XA + XO, 0.1 unit	0.92 ± 0.31		
E	XA + XO, 0.1 unit + DHA, 2.5 mM	1.0 ± 0.5	108	(5)

Paired lenses were incubated as C and E in the medium with additions indicated above.

XA=xanthine, 1 mM.

XO=xanthine oxidase.

n=number of contralateral pairs used in each group of experiments.

AIB=alpha-aminoisobutyric acid.

Rubidium and AIB were used as $^{86}\text{RbCl}$ and $^{14}\text{CAIB}$ respectively.

C=control.

E=contralateral lenses incubated as experimental.

Table 6
The Energetics of Ascorbate Reaction with Xanthine Oxygen Interaction Products
Generated by Xanthine Oxidase (31)

Energetics of the the reactions of ascorbate with ROS (32).

	Δn	E° (Volts)	$\Delta G^{\circ\ddagger}$ (kcal/mol)	Rate Constant
$HA^- \longrightarrow A + H^+ + 2e$		-0.108		
$H_2O_2 + 2H^+ + 2e \longrightarrow 2H_2O$		+2.640		
$HA^{-a} + H_2O_2 + H^+ \longrightarrow A + 2H_2O$		2.532	-58.4	
$HA^- \longrightarrow A^{\bullet-} + H^+ + e$		-0.282		
$OH^\bullet + H^+ + e \longrightarrow H_2O$		+2.180		
$HA^{-b} + OH^\bullet \longrightarrow H_2O + A^{\bullet-}$		1.898	-43.8	$1.3 \times 10^{10} M^{-1}s^{-1}$
$HA^{-c} + O_2^- \longrightarrow \text{products}$			-15.2	$5 \times 10^4 M^{-1}s^{-1}$

Table 7
UV-B induced damage to lens. Prevention by caffeine

Prevention of UVB induced biochemical damage to the lens by caffeine in vitro. Lenses incubated in Tyrode pulsed with $^{86}\text{RbCl}$ were for a 5 hr. period in dark and exposed UV at 300nm and then analyzed for the uptake of rubidium and the contents of ATP and GSH (50)

	Dark Control	UV	UV +Caffeine
CL/CM	29 $\pm$ 3	17 $\pm$ 4	28 $\pm$ 2.5
ATP ($\mu\text{moles/g}$)	2.4 $\pm$ 0.3	0.9 $\pm$ 0.3	2.9 $\pm$ 0.25
GSH ($\mu\text{moles/g}$)	3.8 $\pm$ 0.3	1.2 $\pm$ 0.4	3.5 $\pm$ 0.2

Table 8
Prevention of iron induced damage to lens in culture by caffeine

Prevention of iron-induced damage to the lens in culture by caffeine. Lenses were incubated in dark in the absence and presence of Fe_8Br_8 in the absence and presence of caffeine and analyzed as described under table 7 (51).

	Control	Fe_8Br_8	Fe_8Br_8 + Caff
CL/CM	23 ± 2	7.2 ± 4	14.6 ± 4
ATP ($\mu\text{moles/g}$)	0.96 ± 0.1	0.3 ± 0.2	0.85 ± 0.35
GSH ($\mu\text{moles/g}$)	1.32 ± 0.46	0.62 ± 0.3	1.2 ± 0.4

Table 9
Protective effect of Tempol against iron-induced lens damage

Protective effect of Tempol against iron induced oxidative damage to the lens. Experiments were done as described under Table 8 (51).

	Control	Fe ₈ Br ₈	Fe ₈ Br ₈ + Tempol
CL/CM	38.3 ± 0.35	20 ± 4.6	40.5 ± 0.5
ATP (μmoles/g)	3.5 ± 1	2.6 ± 0.7	3.7 ± 0.4
GSH (μmoles/g)	3.4 ± 0.4	2.2 ± 0.4	3.1 ± 0.75

Table 10
Prevention of kynurenine induced photodamage to the lens by caffeine

Preventive effect of caffeine against Kynurenine dependent UVA damage to lens. Lenses were incubated in medium 199 containing 0.5mM Kynurenine in the absence and presence of UVA adjusted to 1mW/sq.Cm. (52).

	Dark control	UVA + Kyn	UVA + Kyn + Caffeine
CL/CM	33.6 ± 2	6.8 ± 3.5	28 ± 5
ATP (μmoles/g)	0.66 ± 0.15	0.2 ± 0.08	0.6 ± 0.2
GSH (μmoles/g)	1.35 ± 0.12	0.24 ± 0.2	1.4 ± 0.6