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UV-mediated cataractogenesis: a radical perspective

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Abstract. A number of epidemiologic and experimental studies indirectly support the idea that solar ultraviolet radiation may be cataractogenic. However, the physical and cellular processes which might be involved in such cataractogenesis are by no means clear. Because a major consequence of the UV irradiation of oxygenated organic matter is the production of activated oxygen species, the involvement of oxidants has been suspected to be of importance. However, because the lens may normally exist in an hypoxic or even anoxic environment, the extent of availability of oxygen for such reactions is presently unknown. So also are the possible mechanisms through which putative UV damage of the lens might eventuate in cataract. In addition to possible rapid and direct lethal damage to lens epithelium, possible cumulative damage to both lenticular DNA and proteins may occur. Furthermore, UV radiation has the potential to photolytically destroy light-sensitive nutrients and to generate damaging oxidants through interaction with ferruginous compounds. Given that Nature has probably provided the lens with substantial protective devices to ward off damaging effects of UV light, it is still an open question as to whether solar radiation contributes to cataract formation and, if so, by what mechanisms.

This brief review addresses the question of whether solar ultraviolet (UV) radiation, especially at higher energy wavelengths below 320 nm (UV-B), may cause cumulative lenticular damage through radical-based mechanisms. The likelihood that this occurs devolves upon two questions. First, how much UV-B actually reaches the lens? Second, if significant amounts of UV-B do reach the lens, what components of the lens are likely damaged and by what reactions?

With respect to the question of how much UV-B reaches the lens, the cornea and the aqueous humor combined absorb almost all incident radiation at wavelengths below 300 nm. Nonetheless, significant amounts of UV-B at wavelengths above 300 nm may successfully penetrate both the cornea and the aqueous humor, although practically no UV-B will traverse the entire lens [1–3]. Therefore, given the substantial absorptive capacity of the lens itself, the area most likely affected would be the anterior portion. The pattern of transmittance of UV light by the cornea and aqueous humor accords well with the action spectrum published many years ago by Bachem [2] for UV-mediated generation of cataract in guinea pigs and rabbits. The relative cataractogenic effect of UV falls off drastically below 300 nm. So, it is conceivable that sufficient UV, at least at wavelengths > 300 nm, may penetrate the barriers of atmosphere, cornea and aqueous humor to affect the lens. Furthermore, the work of Bachem [2] and several others (e.g., Jose [4]) affirms

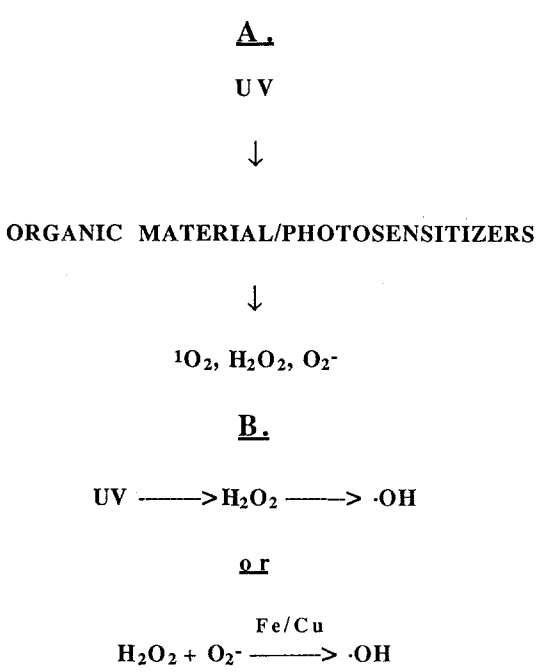

Fig. 1. Activated oxygen species which may form upon UV irradiation of water containing organic material (A) and consequent generation of the dread hydroxyl radical (•OH) by UV-driven homolysis of H_2O_2 or by metal-driven reactions (B).

that animals subjected to UV radiation (and isolated lenses exposed to substantial fluences of UV-B radiation (e.g., Hightower and McCready [5])) may accumulate damage which resembles authentic cataract.

As a result of UV absorbance by the lens, what is the potential for occurrence of damaging oxidative events? When pure water is exposed to UV radiation, practically no activated oxygen arises because pure water cannot absorb the energy [6]. However, as shown diagrammatically in Fig. 1, UV irradiation of water containing organic material – like oceans, lakes or ocular material – will generate activated oxygen species such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and singlet oxygen ($^1O_2$). Furthermore, the dread hydroxyl radical (•OH) may be secondarily produced by UV homolysis of H_2O_2 or via metal catalyzed-reactions. Both •OH and $^1O_2$ (which can be generated via UV reactions with photosensitizers) have the potential to cause substantial damage to biological material.

The importance of oxygen in the damaging effects of UV radiation is readily apparent in the fact that, in the presence of oxygen, UV is much more lethal. As shown in Fig. 2, the killing of *E. coli* by UV at various wavelengths is consistently about one log more effective in the presence of

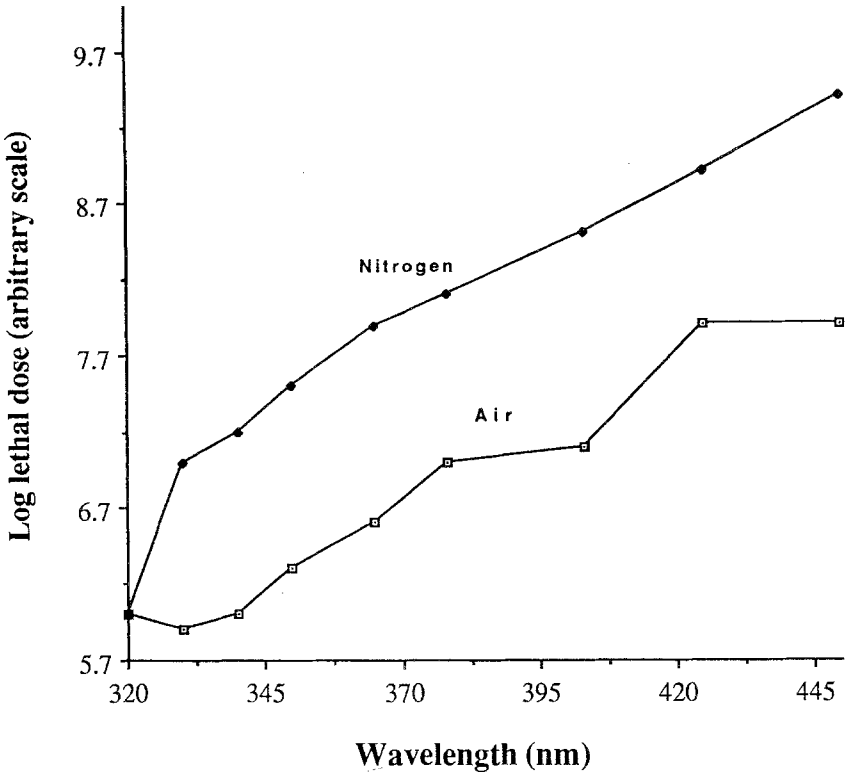

Fig. 2. Oxygen (air) sensitizes *E. coli* to killing by near-UV irradiation. Note the consistent one log incr ment in lethal UV dose in the absence of oxygen. Roughly redrawn from Webb and Brown [7]. The reader is encouraged to consult the original publication for more precise rendition of these results.

air [7]. The reasons for the synergism between oxygen and UV radiation are not completely clear, but one contributory factor may be the coordinate production of H_2O_2 which, as just shown, can arise from UV radiation of organic material. The damaging effect of H_2O_2 and UV radiation are, in at least some systems, synergistic [8]. Similarly, the killing of mammalian cells as promoted by singlet oxygen-generating sensitizers is usually dependent on the presence of oxygen (e.g., [9]), obviously a requisite co-factor for such agents. Overall, then, it appears that oxygen powerfully synergizes the UV-induced killing of cells.

The proximate cause of UV killing of cells in experiments such as these is not fully known. It is probable that simple oxidants such as H_2O_2 and superoxide are not too important in this damage. For example, catalase-deficient *E. coli* do not have greatly enhanced sensitivity to near-UV radiation when exposed under aerobic conditions [10], nor are bacteria with very high

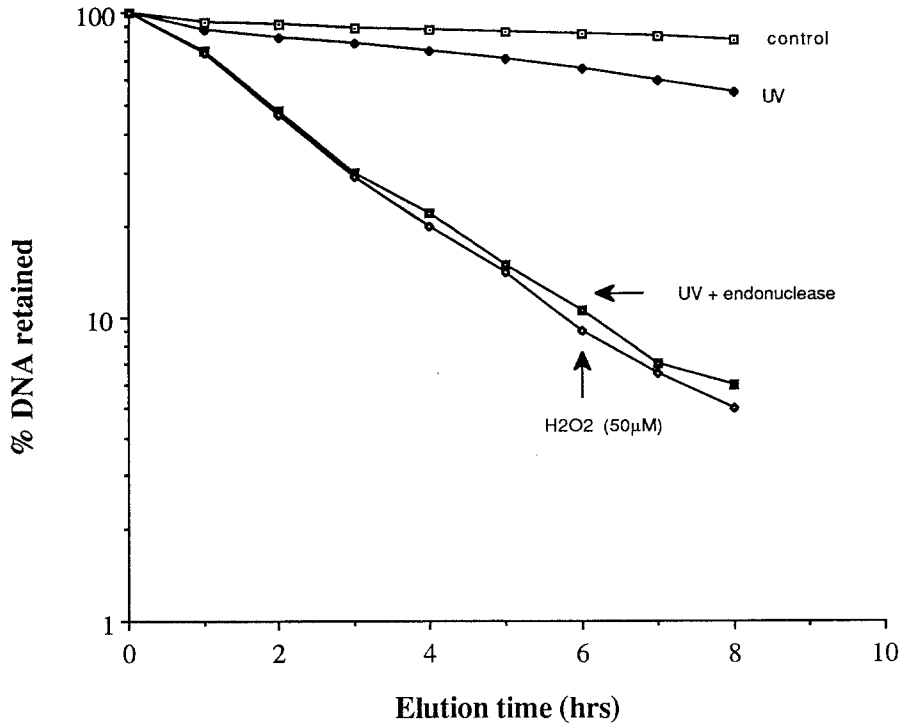

Fig. 3. Clastogenic effects of H_2O_2 vs. UV-B on cultured bovine lens epithelium. DNA, extracted from bovine lens epithelium following exposure to either H_2O_2 (50 μM) or UV-B irradiation. Single strand breaks are revealed by decreased retention of DNA. Note that whereas micromolar H_2O_2 caused substantial numbers of breaks, UV-B irradiation did not, except when samples were subsequently treated with a bacteriophage T4 endonuclease V which cleaves DNA at pyrimidine dimers. Roughly redrawn from Kleiman *et al.* [11]. The reader is encouraged to consult the original publication for a more precise rendition of these results.

superoxide dismutase activity particularly resistant to UV. In the case of mammalian cells, the important damage at minimally lethal doses of UV may be to DNA. A most frequent type of UV-mediated DNA damage is the formation of pyrimidine dimers. There are mechanisms for the repair of these dimers, but these are not infallible.

DNA damage has been observed by Kleiman and colleagues in cultured lens epithelium exposed to UV-B [11]. As shown in Fig. 3, DNA extracted from normal lenticular epithelium shows almost no strand breaks. In contrast, DNA from epithelium exposed to H_2O_2 has extensive breaks. Surprisingly, UV exposure causes very few breaks, indicating that DNA damage – to the extent that it occurs – does not arise from peroxide produced by the irradiation. However, if the DNA extracted from UV-irradiated cells is treated with a bacterial endonuclease capable of incising pyrimidine dimers, large num-

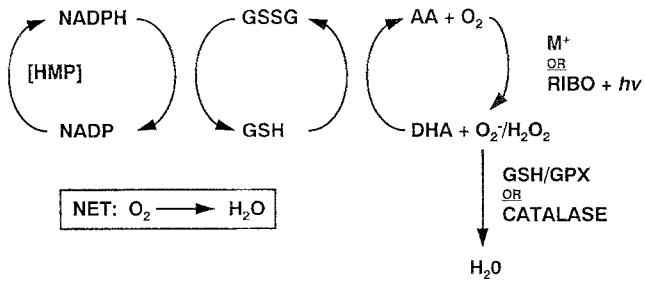

Fig. 4. Reactions whereby ascorbic acid (AA) may react with oxygen (in reactions facilitated by transition metals (M+) or riboflavin and UV light), producing dehydroascorbate (DHA), partially reduced oxygen species and, ultimately, water. The DHA may then be reduced back to AA by reduced glutathione (GSH) or (not shown) NAD(P)H-dependent reactions. Oxidized glutathione (GSSG) is then reduced to GSH employing NADPH generated by the hexose monophosphate pathway (HMP).

bers of breaks appear indicating that extensive pyrimidine dimer formation occurred during the radiation. The importance of this type of DNA damage is emphasized by consideration of humans with rare defects in repair of UV damage to DNA. Cells from such patients are readily killed by ordinarily innocuous doses of UV.

This leads to the proposition that, if the clastogenic effects of UV on lens epithelium are important in UV-associated cataract, then patients with congenital sensitivity to UV-induced DNA damage should have increased frequency of cataract. Indeed, patients with Cockayne syndrome (who have normal excision repair of pyrimidine dimers but exhibit a block in DNA replication following exposure) have a high frequency of cataract [12, 13]. However, the cataract often has a very early onset, and may be independent of UV exposure. Furthermore, patients with xeroderma pigmentosum (who do have defective excision repair) are not remarked to have frequent cataract [14] (although cataracts have been reported in one particular variant of xeroderma [15]). On the other hand, such patients probably spend little time in the sun. So, the evidence is inconclusive.

Other, non-DNA type damage has also been invoked to explain UV-driven cataractogenesis. This would likely involve UV-mediated protein cross-linking or progressive membrane damage arising from either oxidative or non-oxidative pathways. To the extent that oxidative processes are involved, one might expect patients with impaired oxidant defense (such as acatalasemia or glucose-6-phosphate dehydrogenase deficiency) to have increased frequency of cataract. Evidence on both sides has been reported for G-6-PD deficiency. Two recent papers indicate a lack of relationship with the more frequent variants of G-6-PD deficiency [16, 17] whereas scattered earlier reports suggest an association between G-6-PD deficiencies and cataract

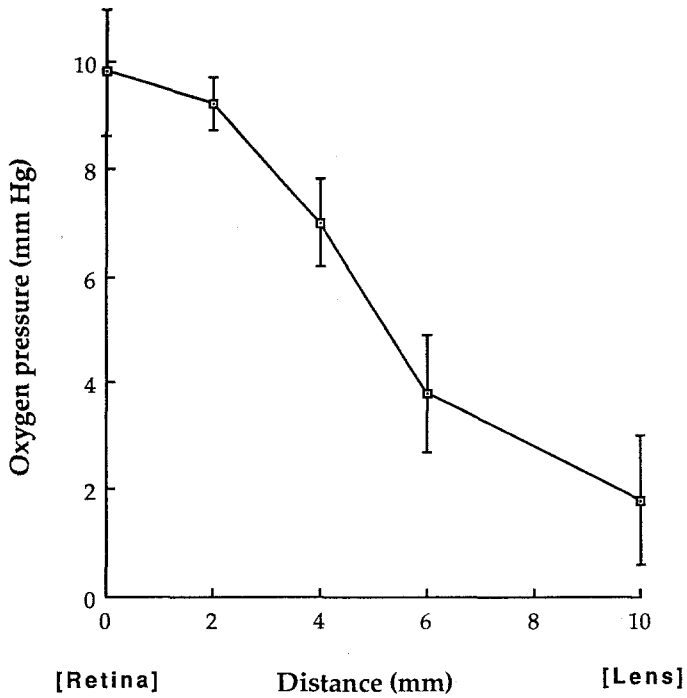

Fig. 5. Decline in oxygen tension during transit of an oxygen electrode through the vitreous humor from the retina to the posterior aspect of the lens in intact rabbit eyes. Data represent average values derived from six animals and vertical lines denote ± 2 S.E.M. Redrawn from Omerod *et al.* [24]. The reader is encouraged to consult the original publication for a more precise rendition of these results.

[18–20]. I know of no reports of cataract which might be UV-induced in the rare humans with acatalasemia [21]. This ignores, of course, a number of experimental animal studies in which cataract has been reported following pharmacologically-induced impairment of catalase and glutathione systems. However, these experimental studies rarely have employed UV as an additional cataractogenic stimulus. Once again, the evidence from these patients with congenital defects in oxidant defense is inconclusive.

In fact, to the extent that UV might act through partially oxidative mechanisms, it is necessary that oxygen should be present in the lens. This may not be the case. It has been argued by one investigator (with questionable credentials in this area) that the lens may be either anoxic or severely hypoxic by design [22]. That is, the very high levels of ascorbic acid normally present in the aqueous and vitreous humors of diurnal mammals may function to convert incoming oxygen to water. This may occur through coupled oxidation of ascorbate, perhaps involving either light or metal catalyst. As long

as the resulting H_2O_2 is efficiently metabolized, the net effect of these reactions is the conversion of oxygen to water (Fig. 4). In one simple example of ascorbate-induced deoxygenation, micromolar amounts of copper added to millimolar concentrations of ascorbic acid will cause total deoxygenation of a solution within a few minutes. Unadulterated humor – both aqueous and vitreous – will cause similar but less rapid oxygen consumption when supplemented with ascorbate [22]. In fact, Wolff *et al.* [23] have shown an equally brisk ascorbate- and riboflavin-dependent oxygen consumption which occurs when the mixture is UV irradiated.

Because the lens is avascular, such reactions could create an oxygen gradient from the retina and the cornea showing steep decline as one approaches the lens. In fact, there are some direct determinations of intraocular oxygen tension which support this idea (e.g., [24, 25]). As shown in Fig. 5, as a micro-oxygen electrode moves through the vitreous humor from the retina to the posterior of the lens, the oxygen tension approaches zero. (It should be mentioned that not all investigators have reported lens oxygen tensions this low.) Therefore, one very important defense employed by the lens against UV damage may be in excluding oxygen which will otherwise synergize damage done by UV radiation. If so, lenticular UV damage may not involve the collaboration of oxygen or of typical oxidative reactions, although DNA damage – much of which can occur without the involvement of oxygen – may certainly play a role.

What other kinds of mischief might UV light cause in the eye which would contribute to cataractogenesis? There would seem to be at least two additional possibilities. First, like any other tissue, the lens epithelium requires adequate levels of certain vitamins and nutrilites for normal growth and differentiation. Two vitamins of particular importance to growing cells – folate and vitamin B12 – are quite photosensitive. Folate, for example, is readily photolyzed by cleavage between the pterin ring and the para-amino benzoic acid moiety. *In vitro*, photolytic destruction of folate is readily demonstrated by the progressive decline in folate in serum exposed to a simple black light. In fact, in mice which were shaved and given radiolabeled folate, UV exposure caused accelerated loss of the vitamin (even though the skin is relatively impervious to most UV light) [26]. Therefore, one possibly noxious result of UV radiation which has been relatively ignored is that of the destruction of photosensitive nutrients rather than any direct damage to the lens.

A second type of reaction which has not been widely considered involves the effects of light on material to which transition metals such as iron are bound. An interesting model reaction of this sort has recently been described [27]. The investigators employed several normally stable iron chelates including ferric:EDTA. This particular chelate absorbs well in the mid-UV region.

Consonant with this adsorption of UV light, reduced iron is generated in large amounts (even though the iron in the original chelate is oxidized). This evidently occurs through reactions involving the absorption of UV energy by the chelate, subsequent destabilization of bonds in the organic chelator and donation of an electron by the EDTA to the iron (thereby reducing the iron and fragmenting the EDTA – one product of which, formaldehyde, is readily detected in these reactions). In fact, EDTA:iron can support a light-driven Fenton reaction in which the production of hydroxyl radical is actually driven by UV radiation in the presence of added H_2O_2 [27].

Why should we care about this arcane chemistry? Well, the lens epithelium is known to contain significant amounts of iron [28, 29] in storage proteins such as ferritin and in heme enzymes such as catalase. UV-A radiation of ferritin has been shown by other investigators to cause the release of ferrous iron as just described for the EDTA:iron chelate [30]. Furthermore, a paradoxical increase in UV sensitivity has been noted in cells having increased catalase [10, 31], suggesting that catalase (known to be inactivated by UV [32]) is another possible source of reactive iron. Regardless of the source, iron released by UV-catalyzed reactions could inflict substantial damage on the lens, particularly in the presence of H_2O_2 – widely reported to be present in both aqueous and vitreous humors [33, 34].

In conclusion, higher energy UV radiation mediates a large number of reactions which have the potential to damage lenticular epithelium, perhaps eventuating in cataracts. These include the production of activated oxygen species, photochemical reactions (oxidative and otherwise) involving transition metals or photosensitizers, and even the possible photolytic destruction of important nutrients. If UV-mediated cataract does occur in humans, given this bewildering variety of possible damaging reactions, the proximate causes are by no means clear. On the other hand, the lens has a number of biologically clever protection devices, including UV absorbers, reducing substances and very low oxygen tension. Does relentless UV radiation overwhelm these protective systems? It may take some time to sort this out.

References

1. Kinsey VE. Spectral transmission of the eye to ultraviolet radiations. Arch Ophthalmol 1948; 39: 508–13.
2. Bachem A. Ophthalmic ultraviolet action spectrum. Am J Ophthalmol 1956; 41: 969–80.
3. Parrish JA, Anderson RR, Urbach F, Pitts, D. UV-A: Biological effects of ultraviolet radiation with emphasis on human responses to long-wave ultraviolet. New York: Plenum Press, 1978.
4. Jose JG. Posterior cataract induction by UV-B radiation in albino mice. Exp Eye Res 1986; 42: 11–20.

5. Hightower K, McCready J. Mechanisms involved in cataract development following near-ultraviolet radiation of cultured lenses. Curr Eye Res 1992; 11: 679–89.
6. Faust BC, Anastasio C, Allen JM, Arakaki T. Aqueous-phase photochemical formation of peroxides in authentic cloud and fog waters. Science 1993; 260: 73–75.
7. Webb RB, Brown MS. Action spectra for oxygen-dependent and independent inactivation of *Escherichia coli* WP2x from 254 to 460 nm. Photochem Photobiol 1979; 29: 407–409.
8. Ananthaswamy HN, Hartman PS, Eisenstark A. Synergistic lethality of phage T7 by near-UV radiation and hydrogen peroxide: an action spectrum. Photochem Photobiol 1979; 29: 53–56.
9. Zoeller RA, Olivier OH, Raetz CRH. A possible role for plasmalogens in protecting animal cells against photosensitized killing. J Biol Chem 1988; 263: 11590–96.
10. Eisenstark A, Perrot G. Catalase has only a minor role in protection against near-ultraviolet radiation damage in bacteria. Mol Gen Genet 1987; 207: 68–72.
11. Kleiman NJ, Wang RR, Spector A. Hydrogen peroxide-induced DNA damage in bovine lens epithelial cells. Mut Res 1990; 240: 35–45.
12. Nance MA, Berry SA. Cockayne syndrome: review of 140 cases. Am J Med Genet 1992; 42: 68–84.
13. Traboulsi EI, DeBecker I, Maumenee IH. Ocular findings in Cockayne syndrome. Am J Ophthalmol 1992; 114: 579–83.
14. Cleaver JE, Kraemer KH. Xeroderma pigmentosum. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. Vol. II, Sixth Ed. New York: McGraw-Hill, 1989: 2949–71.
15. Rebora A, Crovato F. PIBI(D)S syndrome: Trichothiodystrophy with xeroderma pigmentosum (group D) mutation. J Am Acad Dermatol 1987; 16: 940–47.
16. Bhatia RP, Patel R, Dubey B. Senile cataract and glucose-6-phosphate dehydrogenase deficiency in Indians. Trop Geogr Med 1990; 42: 349–51.
17. Meloni T, Carta F, Forteleoni G, Carta A, Ena F, Meloni GF. Glucose-6-phosphate dehydrogenase deficiency and cataract of patients in northern Sardinia. Am J Ophthalmol 1990; 110: 661–64.
18. Cohn J, Carter N, Warburg M. Glucose-6-phosphate dehydrogenase deficiency in a native Danish family. Scand J Haematol 1979; 23: 403–409.
19. Westring DN, Pisciotta AV. Anemia, cataracts and seizures in a patient with glucose-6-phosphate dehydrogenase deficiency. Arch Intern Med 1966; 118: 385–90.
20. Moro F, Gorgone G, Li-Volti S, Cavallaro N, Faro S, Curreri R, Mollica F. Glucose-6-phosphate dehydrogenase deficiency and incidence of cataract in Sicily. Ophthalmic Paediatr Genet 1985; 5: 197–203.
21. Eaton JW. Acatalasemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. Vol. II, Sixth Ed. New York: McGraw-Hill, 1989: 1551–61.
22. Eaton JW. Is the lens canned? Free Rad Biol Med 1991; 11: 207–13.
23. Wolff SP, Wang GM, Spector A. Pro-oxidant activation of ocular reductants. 1. Copper and riboflavin stimulate ascorbate oxidation causing lens epithelial cytotoxicity *in vitro*. Exp Eye Res 1987; 45: 777–89.
24. Omerod CD, Edelstein MAC, Schmidt GL, Juarez RS, Finegold SM, Smith RE. The intraocular environment and experimental anaerobic bacterial endophthalmitis. Arch Ophthalmol 1987; 105: 1571–75.
25. Barr RE, Roetman EL. Oxygen gradients in the anterior chamber of anesthetized rabbits. Invest Ophthalmol 1974; 13: 386–89.
26. Branda RF, Eaton JW. Skin color and nutrient photolysis: An evolutionary hypothesis. Science 1978; 201: 625–26.
27. Van der Zee J, Krootjes BBH, Chignell CF, Dubbelman TMAR, Van Steveninck J. Hydroxyl radical generation by a light-dependent fenton reaction. Free Rad Biol Med 1993; 14: 105–13.
28. Garland D. Role of site-specific, metal-catalyzed oxidation in lens aging and cataract: A hypothesis. Exp Eye Res 1990; 50: 677–82.

29. McGahan MC. Does the lens serve as a sink for iron during inflammation? Exp Eye Res 1992; 54: 525–30
30. Aubailly M, Santus R, Salmon S. Ferrous iron release from ferritin by ultraviolet-A radiations. Photochem Photobiol 1991; 54: 769–73.
31. Kramer GF, Ames BN. Oxidative mechanisms of toxicity of low-intensity near UV-light. J Bacteriol 1987; 169: 2259–66.
32. Cheng E, Kellog EW, Packer L. Photoinactivation of catalase. Photochem Photobiol 1981; 34:125–29.
33. Bhuyan KC, Bhuyan DK. Regulation of hydrogen peroxide in eye humors. Effect of 3-amino-1H-1,2,4-triazole on catalase and glutathione peroxidase of rabbit eye. Biochim Biophys Acta 1977; 497: 641–45.
34. Giblin FJ, McCready JP, Kodama T, Reddy VN. A direct correlation between the levels of ascorbic acid and H_2O_2 in aqueous humor. Exp Eye Res 1984; 38: 87–93.

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