Specific immunohistochemical localization of superoxide dismutase in ocular tissue of Lewis rats revealed the presence of this enzyme predominantly in the corneal epithelium, endothelium, apical regions of the posterior epithelium of the iris, nonpigmented inner ciliary epithelium, lens epithelium, inner segments of photoreceptor cell layer of the retina, and retinal pigment epithelium. This enzyme is distributed primarily in those ocular structures which may be frequently exposed to superoxide or its metabolites under physiologic conditions and in such pathologic processes as intraocular inflammations. It thus appears that superoxide dismutase and other antioxidants may play a protective role in preventing the damaging effects of oxygen radicals. Invest Ophthalmol Vis Sci 26:1778-1781, 1985

The balance between the production and catabolism of oxidants by cells and tissue is critical for maintenance of the biologic integrity of the tissue. Ocular tissues and plasma contain antioxidants that prevent damage from excessive oxygen metabolites. There are conventional and unconventional antioxidants. Conventional antioxidants act by either peroxide decomposition or by the trapping of radicals and interference with the chain of oxidation reaction. The former antioxidants are called preventive antioxidants, and the latter are known as chain breaking antioxidants. The unconventional antioxidants act by a variety of mechanisms. In this preliminary study of the ocular distribution of antioxidants, we selected a chain breaking antioxidant, superoxide dismutase, whose antioxidant effects in the eye have been the most thoroughly studied to date.

Protection of the lens, cornea, and retina by antioxidants against oxygen radicals formed by photoperoxidation has been demonstrated by several investigators. Superoxide dismutase (SOD) has been shown to inhibit lipid oxidation and prevent cataract formation, and it may protect retinal membrane phospholipids from oxidative alteration by free radicals.

The prevalence of SOD is known from biochemical assays to vary in different ocular tissue, but the specific cell types containing an abundance of this enzyme in ocular tissues remain largely undetermined. Results of various biochemical analyses are difficult to compare since SOD activity is produced by at least three different molecules, including a cytoplasmic copper-zinc SOD, a mitochondrial manganese SOD, and an extracellular SOD of high molecular weight. Specific cytochemical localization of one or another of these enzymes could provide more precise information concerning their subcellular distribution and their relative abundance and distribution in various ocular structures, thus providing knowledge concerning the physiologic significance of the enzyme activity.

Materials and Methods. Six enucleated globes from adult Lewis rats weighing about 250 g (conforming to the ARVO Resolution on the Use of Animals in Research) were fixed in 4% formalin buffered with 2% calcium acetate. The globes were fixed for 6 hr at room temperature. A segment of the globe was removed, and the tissues were dehydrated through a series of graded alcohols, embedded in paraffin, and 5-μm thick sections prepared. Following deparaffination and rehydration, the sections were treated with 3% H₂O₂ for 10 min to inactivate endogenous peroxidase activity of erythrocytes, then washed for 5 min in 0.1 M phosphate buffered saline (PBS), pH 7.2. At 4°C the sections were exposed overnight to anti-rat Cu-Zn SOD at a final dilution of 1:800. This antibody was raised in rabbit by repeated intradermal and intramuscular injections of purified rat Cu-Zn SOD (purified by the method of Crapo and McCord) in complete Freund’s adjuvant using a method similar to that described by Thaete et al.

For controls, additional sections of the globe were treated as above, except for the replacement of rabbit anti Cu-Zn SOD serum with pre-immune serum from the same rabbit that generated the antibody. All sections were washed in PBS for 30 min and incubated for 1 hr at room temperature in biotinylated goat anti-rabbit IgG. After an additional 15-min wash in PBS, the sections were incubated in avidin-biotin-peroxidase complex (ABC-Vector Labs; Burlingame, CA); this was followed by development of 3-amino-9-ethyl carbazol (AEC) H₂O₂ substrate reaction. Several of these sections were counter-stained with hematoxylin.
Zn SOD was consistently localized in the corneal epithelium (Fig. 1), endothelium, iris and ciliary epithelial layers, anterior and equatorial regions of the lens cortex and epithelium, inner segments of the retina and plexiform layers, and retinal pigment epithelium. No immunostaining was present in adjacent cytochemical control sections.

In the corneal epithelium, the basal and intermediate cells exhibited intense intracellular staining, while the superficial epithelial cells near the limbus were totally devoid of immunoreactive SOD. The endothelial cells also showed intracytoplasmic staining. In the iris the posterior epithelial layer, and in the ciliary body the inner ciliary epithelium contained intense immunoreactive SOD (Fig. 2); most of the product appeared to be present in the apical portion of these cells. Even though there was mild reaction from the immunoreactive SOD product distributed within the various layers of the retina, intense reaction was noted within the inner segments and the plexiform layers (Fig. 3) and in the retinal pigment epithelium.

Discussion. The primary biological role of the enzyme superoxide dismutase is to scavenge the superoxide radical (\( \cdot \text{O}_2 \)), a free radical species considered of primary importance in oxygen toxicity because of its ability to form deleterious oxygen intermediates. Cellular damage inflicted by these free radicals includes enzyme inactivation and disruption of lipid membranes.\(^8\)

The presence of superoxide dismutase in the corneal epithelium and in the endothelium suggests its possible protective role in preventing damage from photochemical generation of superoxide and other potent oxidants. The aqueous humor and the lens, by virtue of their own transparency as well as the transparency of the cornea, offer a unique opportunity for in vivo photochemical generation of superoxide and other injurious radicals. Varma et al\(^3\) have demonstrated that
the intraocular photocatalytic generation of oxygen radical was harmful to the lens, as indicated by the formation of excessive lipid peroxides. Superoxide dismutase appears to play a role in preventing damage from this photocatalytic generation of oxygen radicals through its distribution in the endothelium of the cornea, iris and ciliary epithelium, and in the lens, as these structures are constantly exposed to the aqueous humor and its photocatalytic products. In pathologic conditions such as anterior uveitis, the inflammatory cells could produce superoxide and its metabolites. In such conditions the presence of the enzyme SOD in the lining cells of ocular cavities may minimize the destructive effects of these metabolites on the endothelium, iris and ciliary processes. Similar beneficial effects may be operative in the vitreous by preventing superoxide-mediated depolymerization of hyaluronic acid.

Hall and Hall have reported the possible role of SOD in protection against free radical oxidation of the retinal phospholipid membrane. Along with other preventive and chain breaking antioxidants, such as vitamin E, SOD may play a significant role in preventing oxygen toxicity in photochemical generation of superoxide and its injurious intermediate radicals. These investigators demonstrated the presence of SOD enzyme activity predominantly in the isolated outer segments of bovine and frog retina by the chemical nitroblue tetrazolium photoreduction system. The enzyme activity (per microgram of protein) in the bovine and frog outer segment extracts was 200 to 400 times greater than that in the remainder of the retina. Unlike the results using this chemical method, our immunohistochemical localization of this enzyme in rat ocular tissue revealed the presence of SOD mainly in the inner segments of the retina (Fig. 3) and the plexiform layers. This discrepancy could be due to (1) differences in the methodology employed or (2) difference in distribution of this enzyme in various animal species and/or (3) the method of comparison, namely the enzyme activity in outer segments, which was compared with that in the remainder of the entire retina rather than with inner segments.

The significance of SOD distribution selectively in various subcellular structures of the eye can at this time be only speculative. However, precise localization of the enzyme by the immunohistochemical method revealed its presence mainly in those tissues that are predisposed to exposure to oxygen radicals in a physiologic state as well as such pathologic conditions as intraocular inflammations. It thus appears that SOD, along with vitamin C and other antioxidants, plays a protective role in precluding the damaging effects of superoxide radical and its metabolites.

Key words: superoxide dismutase, immunohistochemistry, oxygen radicals, ocular inflammation, antioxidants
**Acknowledgments.** We are grateful to Dr. Rosalie Crouch and to Mary Simpson for their assistance in purifying the rat Cu-Zn SOD used in this project. The authors thank Ana Maria Zaragoza for her technical assistance.

From Estelle Doheny Eye Foundation and Departments of Ophthalmology and Pathology, University of Southern California, Los Angeles, and from the Medical University of South Carolina.* Supported by Estelle Doheny Eye Foundation research funds, in part by Research to Prevent Blindness and by the National Institute of Health grant EYO5662. Submitted for publication: July 25, 1984. Reprint requests: N. A. Rao, MD, Estelle Doheny Eye Foundation, 1355 San Pablo Street, Los Angeles, California 90033.

**References**


**Effect of Oxygen on Aqueous Humor Dynamics in Rabbits**

Michael E. Yablonski,* Pamela Gallia and Douglas Shapirot

A study was made in albino rabbits of the effect on aqueous humor dynamics of 100% oxygen, administered by face mask. A mean decrease in intraocular pressure of 4.9 mm Hg was found. This was accompanied by a decrease in episcleral venous pressure of 4.5 mm Hg. Anterior chamber aqueous humor flow decreased transiently after oxygen administration but returned to pre-oxygen levels after about 60 min. It was concluded that the sustained decrease in intraocular pressure which was caused by oxygen was secondary to the decrease in episcleral venous pressure and not to a decrease in the production of aqueous humor. Invest Ophthalmol Vis Sci 26: 1781–1784, 1985

Administration of 100% oxygen by face mask was shown by Gallin and coworkers1 in humans and albino rabbits to lower intraocular pressure. The mechanism of this action of oxygen was unclear; therefore, the present study was undertaken in rabbits to elucidate the effect of oxygen administration on aqueous humor dynamics. Fluorophotometry was used to measure anterior chamber aqueous humor flow. Tonography was used to measure outflow facility. In addition, episcleral venous pressure was measured.

**Materials and Methods.** In albino rabbits weighing 2.0–2.5 kg, 100% oxygen was administered by face mask as described previously.1 The effect of oxygen on intraocular pressure, anterior chamber aqueous humor flow, episcleral venous pressure, and total outflow facility in unanesthetized rabbits wrapped in cloth restraint was determined. In the case of all measurements the values of the two eyes of the rabbit were averaged and considered representative of one "cycloptic" eye of each rabbit.

Fluorophotometry was done on 20 rabbits using the method of Yablonski and coworkers.2 Each eye was given 0.25% fluorescein and 0.4% HCl benoxinate (Flures, Barnes-Hind; Sunnyvale, CA), 2 drops every 15 min for a total of 4 administrations (8 drops). Four and one half hours after the last fluorescein administration, fluorophotometry measurements were begun. Baseline fluorophotometry measurements were made at 45-min intervals for a total of 4 sets of measurements. One hundred percent oxygen administration was then begun, during which time fluorophotometry measurements were continued at 20-min intervals.

The effect of oxygen administration on episcleral venous pressure was determined by the chamber method.3 The end point was the complete collapse of the vessel. Baseline episcleral venous pressure measurements were made in both eyes of 20 unanesthe-