Oxygen-induced visual cell degeneration in the rabbit

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High oxygen concentrations induced visual cell degeneration in albino and pigmented rabbits. The most sensitive cells were located in the area centralis, a zone analogous to the human macula, and characterized by low glycogen content and rich choroidal blood supply. Electron microscopy demonstrated early pathological changes of intracellular membranes followed by total disorganization of visual cells, particularly those with rod-type synapses. DL-alpha-tocopherol acetate did not prevent visual cell damage.

Key words: retinal photoreceptor cell degeneration, oxygen, visual cells, area centralis, vitamin E, pharmacodynamics, glycogen, concentration, choroidal blood flow, histopathology, rabbits.

High concentrations of oxygen have been shown to exert a deleterious effect on a variety of tissues. The selective susceptibility of the visual cells of the rabbit retina to the toxic effects of oxygen was first reported by Noell, who demonstrated functional changes with electroretinography and structural changes with light microscopy in albino rabbits exposed to high oxygen concentrations. The histological features were characterized by selective degeneration of the visual cell nuclei and the photoreceptors with preservation of the integrity of all other retinal layers. The most vulnerable visual cells were located in the central retina, while those in the immediate peripapillary zone and in the retinal periphery were relatively spared. Within a particular retinal area the rod cells were more sensitive to oxygen poisoning than the cone cells. Furthermore, Noell showed that the visual cells of newborn rabbits were more resistant to oxygen than those of adult rabbits; the increasing susceptibility with age seemed to parallel the structural differentiation and metabolic organization of the visual cells.

The present investigation confirms and elaborates on the light microscopical features of oxygen-induced visual cell degeneration and for the first time describes the ultrastructural features of the degenerative process. The effect of the administration of DL-alpha-tocopherol acetate (vitamin E) on the course of the degenerative process is also reported.
Materials and methods

**Technique of oxygen exposure.** Animals utilized in the study were adult albino and pigmented rabbits weighing between 2 and 3 kilograms. Pairs of animals were placed in an incubator through which oxygen was circulated at a controlled rate of inflow in order to maintain the concentration of oxygen between 98 and 100 per cent at ambient pressure. Carbon dioxide was absorbed with soda lime. The temperature in the tank varied between 22 and 25°C. The duration of exposure in various experiments ranged from 24 to 80 hours with periods of survival in room air after exposure ranging from 0 to 16 days.

**DL-alpha-tocopherol acetate supplementation.** Certain adult albino rabbits maintained on a routine laboratory diet were pretreated with intraperitoneal DL-alpha-tocopherol acetate using various dosage regimens. The doses ranged from 20 to 400 mg per kilogram of body weight administered daily from 2 days to one week prior to oxygen exposure, and then repeated daily during the period of survival. Control animals untreated with DL-alpha-tocopherol acetate, maintained on the same laboratory diet and matched for weight, were included in the same tank during exposure to oxygen.

**Neoprene casts of the choroid.** In order to study the anatomy of the choroidal vasculature in normal and experimental animals, the common carotid artery on one side was cannulated under light pentobarbital (Nembutal) anesthesia. After the jugular vein was severed, the vascular tree was flushed with a solution of sodium nitrite. The vein was then clamped and neoprene latex, diluted 1:3 and colored red with carmine dye, was forcefully injected into the artery with a hand-operated syringe. The homolateral eye was enucleated and rapidly opened by a coronal section through the ora serrata. After removal of the vitreous, the posterior half of the eye was cut into segments and fixed in 2.5 per cent glutaraldehyde in 0.09M sodium cacodylate buffer at pH 7.3 for 2 hours. The retina and choroid were then washed in 0.09M cacodylate buffer in 7.5 per cent sucrose for 1 to 1½ hours and postfixed in 1 per cent isotonic veronal buffered osmium tetroxide at pH 7.3 for 1½ hours. The specimen was then dehydrated in graded concentrations of ethyl alcohol and embedded in epoxy resin (Epon). Sections 1 to 2 μ thick were cut from the whole block and stained with alkaline toluidine blue for study by light microscopy. Ultrathin sections cut from selected areas of the block were stained with 5 per cent uranyl acetate in 1 per cent acetic acid, followed by 0.4 per cent lead citrate in 0.1N sodium hydroxide, and viewed by an A.E.I. EM6 electron microscope.

**Results**

**Fundus examination.** No fundus abnormalities were noted in animals subjected to 100 per cent oxygen for 24 to 40 hours and allowed to survive up to 16 days. One albino rabbit examined 3 months after oxygen exposure showed slight pallor of the retina and choroid in the region of the area centralis. The paucity of fundus findings can be attributed to the mild degree of atrophic and proliferative changes in the pigment epithelium and to the structural preservation of the inner retinal layers to be described below. There was no evidence of retinal detachment or cotton-wool spots as reported after exposure of dogs to high oxygen concentration.6, 7

**Light microscopy.**

**Normal adult rabbit.** The section shown in Fig. 1, A was taken from the central portion of the retina below the optic disc, a region known as the area centralis or visual streak and structurally analogous to the human macula. Although it lacked

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*Potassium dichromate (5 per cent solution) 4 parts; 40 per cent formaldehyde (10 per cent solution) 4 parts; glacial acetic acid, 1 part.

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**Electron microscopy.** For electron microscopical study the animals were lightly anesthetized by intravenous injection of pentobarbital (Nembutal), and the eyes were enucleated and rapidly opened by a coronal section through the ora serrata. After removal of the vitreous, the posterior half of the eye was cut into segments and fixed in 2.5 per cent glutaraldehyde in 0.09M sodium cacodylate buffer at pH 7.3 for 2 hours. The retina and choroid were then washed in 0.09M cacodylate buffer in 7.5 per cent sucrose for 1 to 1½ hours and postfixed in 1 per cent isotonic veronal buffered osmium tetroxide at pH 7.3 for 1½ hours. The specimen was then dehydrated in graded concentrations of ethyl alcohol and embedded in epoxy resin (Epon). Sections 1 to 2 μ thick were cut from the whole block and stained with alkaline toluidine blue for study by light microscopy. Ultrathin sections cut from selected areas of the block were stained with 5 per cent uranyl acetate in 1 per cent acetic acid, followed by 0.4 per cent lead citrate in 0.1N sodium hydroxide, and viewed by an A.E.I. EM6 electron microscope.
Fig. 1. A, Normal adult rabbit retina, section taken from the area centralis. Parts B, through D show the results of various survival periods about 40 hours of 100 per cent oxygen. B, Immediately put to death; photoreceptors with disorganized inner segments; pyknotic nuclei in edematous outer nuclear layer. C, 2 days’ survival; photoreceptors replaced by debris; pyknotic nuclei in attenuated outer nuclear layer. D, 16 days’ survival; single remaining layer of photoreceptor cell nuclei (between the arrows) interspersed with larger migrated nuclei of Müller cells. pr, Photoreceptors; onl, outer nuclear layer. (Hematoxylin and eosin; ×300.)

a central foveal pit, it showed a thickening of the outer and inner nuclear layers and an increased number of ganglion cells.

Throughout most of the normal rabbit retina the radial fibers of the Müller cells were very prominent and showed a high glycogen content as demonstrated by intensely positive staining by the periodic acid–Schiff technique (Fig. 2, A) (PAS-negative after salivary amylase digestion). In contrast, considerably paler staining with PAS was found in the Müller cells of the area centralis indicating a lower glycogen content (Fig. 2, B).

Exposure for 24 to 40 hours to 100 per cent oxygen—immediately put to death. The earliest detectable changes occurred in the photoreceptors and nuclei of the visual cells in the area centralis (Fig. 1, B). Swelling of the inner segments was associated with pyknosis of the visual cell nuclei and edema in the outer nuclear layer. The outer segments of the visual cells were relatively spared at first, but soon the entire photoreceptor showed extensive degeneration. The inner retinal layers were intact and there was no evidence of retinal detachment. There was a generalized depletion of glycogen throughout the retina (Fig. 2, C), including the more peripheral regions which were spared from neuronal degeneration.

Exposure for 40 hours to 100 per cent oxygen—2 days’ survival. In the area centralis the inner and outer segments of the photoreceptors appeared as disorganized debris (Fig. 1, C). Most of the visual cell nuclei showed more advanced pyknosis, and many had disappeared. Occasional larger, pale-staining nuclei found in the outer nuclear layer seemed to be Müller cell nuclei which had migrated from their usual position in the inner nuclear layer. Glycogen had begun to reaccumulate throughout the retina, but was most prominent centrally in the area of greatest neuronal degeneration (Fig. 2 D).

Exposure for 40 hours to 100 per cent oxygen—16 days’ survival. In the area centralis the visual cells were reduced to a single surviving layer of nuclei with no
clearly defined inner and outer segments (Fig. 1, D). Migrated Müller cell nuclei formed a row just external to the outer plexiform layer. The glycogen content in the outer nuclear layer was greatly increased whereas that in the inner retinal layers seemed normal (Fig. 2, E). There was only mild irregularity of the retinal pigment epithelium and the inner retinal layers were intact. Under the experimental conditions described an occasional animal showed some visual cell degeneration a short distance above the optic disc, but the immediate peripapillary region and the periphery of the retina remained uninvolved.

**Electron microscopy.**

*Normal retina.* The ultrastructural features of the normal rabbit retina have been described in detail by Sjöstrand and Nilsson and were confirmed in the present study. Reference to Fig. 3 demonstrates the highlights which relate to the visual cells. The outer nuclear layer was composed of receptor cell nuclei which normally showed prominently clumped chromatin and were surrounded by a double-layered nuclear membrane, enclosing a narrow perinuclear cisterna. The inner segment of the visual cell could be subdivided into a distal ellipsoidal portion, containing large, elongated mitochondria, and a proximal myoid portion with a well-developed endoplasmic reticulum and Golgi complex. The outer segment of the visual cell consisted of densely packed, uniform, membranous lamellae which lay in close apposition to the fingerlike apical processes of the pigment epithelium. Normally, the pigment epithelium had a complex convoluted basal border adjacent to Bruch's membrane, and contained components of the endoplasmic reticulum and a concentration of mitochondria around a large centrally placed nucleus.

*Exposure for 40 hours to 100 per cent oxygen—immediately put to death.* The most striking early degenerative change was the formation of prominent membrane-bound vesicles containing some fine amor-
Fig. 3. Normal adult rabbit retina showing visual cell nuclei (N) in the outer nuclear layer. Photoreceptor outer segments (OS) are connected by ciliary structures to the inner segments (IS) which contain numerous elongated mitochondria (M) and a well-developed endoplasmic reticulum (ER). (x8,750.)

Fig. 4. Adult rabbit retina—immediately put to death after 40 hours’ exposure to 100 per cent oxygen. The vesicles (V) in the inner segments (IS) probably represent swollen endoplasmic reticulum. Some mitochondria (M) show early degeneration of cristae, and others have assumed large bizarre shapes. Note the dilated perinuclear cisterna (PC) surrounding otherwise normal nuclei. (x5,500.)
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phous material and located in the inner segment of the visual cell (Fig. 4). No granules were found lining the membranous walls of the vesicles. On the basis of their structure and distribution the cytoplasmic vesicles most likely represented swollen components of the endoplasmic reticulum and Golgi apparatus, although some of them might have been degenerated mitochondria. The vesicles could be seen distorting adjacent mitochondria by compression, and an occasional mitochondrion had lost the regular orientation of its cristae.

The chromatin pattern and the general architecture of the visual cell nuclei were normal at this stage, but there was a clear halo around many nuclei resulting from the separation of the outer and inner membranes of the nuclear envelope to form a swollen perinuclear cisterna (Fig. 5). In normal cells the nuclear envelope is thought to be a derivative of the endoplasmic reticulum, and continuity of the outermost membrane of the envelope with the membranes of the endoplasmic reticulum has been demonstrated. Therefore, it is not surprising that both elements of this complex intracellular membrane-bound system showed similar swelling in response to a toxic insult.

At this stage of degeneration the outer segments had retained their uniform lamellar structure, even where the inner segment of the same cell showed extensive vesiculation. The focal nature of the degenerative process was also evidenced by normal visual cells lying adjacent to cells with advanced pathological changes.

The synaptic bodies of the visual cells contained a few abnormal vacuoles, but the synaptic vesicles and the rest of the synaptic apparatus were normal (Fig. 6). The inner retinal layers, the pigment epithelium, Bruch’s membrane, and the choroidal vessels all appeared normal. The intimate relationship between the photoreceptor outer segments and the apical processes of the pigment epithelium was preserved.

**Exposure for 40 hours to 100 per cent oxygen—2 days’ survival.** At this stage there was marked disorganization of the entire visual cell in the involved area (Fig. 7). The outer segments appeared as fragmented, rounded, vesiculated bodies of increased electron density. Connections of most of these bodies with the inner segments could not be established. The inner segments were also distorted and contained abnormal mitochondria with a dark homogeneous appearance and irregular cristae. As the visual cell nuclei deteriorated, they decreased in size and showed pyknosis of their nuclear chromatin. The cytoplasm of the Müller cells expanded among the pyknotic visual cell nuclei. Within this cytoplasmic matrix was an abundance of electron-dense granules, radially oriented microfilaments, and aggregates of rough-surfaced endoplasmic reticulum (Fig. 8).

The adjacent pigment epithelium contained multiple, large, and irregular electron-dense bodies, which probably represented ingested visual cell debris. Occasional laminated myeloid figures were also seen within pigment epithelial cells (Fig. 9). The mitochondria of the pigment epithelium were normal in structure but appeared more numerous. Bruch’s membrane was intact, and the choriocapillaris, with its typically fenestrated endothelial lining, was patent.

**Exposure for 40 hours to 100 per cent oxygen—16 days’ survival.** By this stage the debris of degenerated visual cells was scanty (Fig. 10). The few surviving visual cells appeared to possess fairly normal mitochondria and intact nuclei, but the general architecture of the inner segment was compressed and distorted, due most likely to the loss of structural support usually provided by the neighboring visual cells. No outer segments could be identified. Associated with the elements of the surviving visual cells was found an occasional macrophage containing aggregates of electron-dense material (Fig. 11).

Wherever possible the synaptic bodies of the surviving visual cells in the degene-
The intracytoplasmic vesicles (V) contain some fine amorphous material. Arrows point to the inner and outer membranes lining the dilated perinuclear cisterna (PC). (x12,000.)

Fig. 6. Same animal as in Fig. 4, showing the synaptic spherules (S) containing a few large, abnormal vesicles. The synaptic ribbon (arrow) and the elements of the outer plexiform layer (opl) appear normal. (x18,360.)
Fig. 7. Adult rabbit retina (albino)—2 days after 40 hours' exposure to 100 per cent oxygen—showing disorganized outer segments (OS) and inner segments (IS) and pyknotic visual cell nuclei (N). Some of the rounded, vesiculated outer segments are seen to be intimately associated with the pigment epithelium (PE). Choriocapillaris (C). (×6,000.)
Fig. 8. Same animal as in Fig. 6, showing pyknotic visual cell nuclei and degenerated synaptic bodies (S) with abnormal vesicles containing myeloid figures. Arrows point to the limiting membranes of the visual cells. The expanded Müller cell cytoplasm contains aggregates of rough-surfaced endoplasmic reticulum. (×20,000.)
Fig. 9. Same animal as in Fig. 6, demonstrating bizarre, electron-dense bodies bound by membranes (arrow) and myeloid figures (MF). The nucleus and mitochondria and the convoluted basal border of the pigment epithelium appear normal. The degenerated outer segments (OS) are associated with ill-defined apical processes of the epithelium. Note the red blood cell in the patent lumen of the choriocapillaris (C). (xl4,000.)
Fig. 10. Adult rabbit retina (albino)—10 days after 40 hours exposure to 100 per cent oxygen—showing advanced degeneration of the visual cells with 2 surviving visual cell nuclei (N1, N2). The area usually occupied by the photoreceptors is represented by the narrow zone between the arrows. The focal collection of mitochondria (M) belongs to a distorted inner segment. The pigment epithelium (PE) contains some pigment granules and phagocytosed electron-dense debris (D). Expanded Müller cell cytoplasm containing aggregates of rough-surfaced endoplasmic reticulum (rER) occupies most of the outer retinal area. Migrated Müller cell nuclei (N3). Microvillous processes (mv). (×8,500.)
Fig. 11. Same animals as in Fig. 10, showing two visual cell nuclei (N₁, N₂) and inner segments containing degenerated mitochondria. The synaptic body (S) of the visual cell is the complex beta-type with multiple synaptic ribbons (arrows). At the top of the figure is a macrophage (Ma) with ingested electron-dense debris. Migrated Müller cell nucleus (N₃). (x11,000.)
Fig. 12. Same animal as in Fig. 10, demonstrating 3 nuclei in the outer nuclear layer. N₁ and N₂ are visual cell nuclei with complex beta-type synaptic bodies. The central nucleus (N₃) is a migrated Muller cell nucleus surrounded by cytoplasm containing abundant granules, probably mostly glycogen. (x13,000.)

rated area centralis were examined in detail. Sjöstrand and Nilsson have described two types of receptor cell synaptic bodies in the normal rabbit retina: (1) the alpha-type synaptic spherule, which has a single synaptic ribbon and is thought to be characteristic of rodlike cells, and (2) the beta-type synaptic pedicle, which has a higher degree of complexity and multiple synaptic ribbons and is thought to be characteristic of conelike cells. In the normal rabbit the alpha-type synaptic bodies far outnumbered the beta-type synaptic bodies in the area centralis. In contrast, the majority of surviving visual cells found in the area centralis 16 days after oxygen exposure possessed the complex beta-type synaptic pedicle (Fig. 12), suggesting that it was primarily rodlike cells with alpha-type synaptic spherules that had degenerated. One cannot exclude the possibility, however, that the surviving synaptic bodies formed multiple new synaptic connections with elements in the outer plexiform layer as neighboring visual cells degenerated.

Among the surviving visual cell nuclei were interspersed somewhat larger nuclei with more finely clumped nuclear chromatin, which could be identified as migrated nuclei of Muller cells (Fig. 12). The expanded Muller cell cytoplasm surrounding the nuclei occupied the major portion of the outer retinal region in the degenerated area centralis. The abundance of rough-surfaced endoplasmic reticulum concentrated in the Muller cell cytoplasm indicated an active protein synthesis associated with the great increase in cytoplasmic matrix. No other type of glial cell could be found taking part in the reparative process. These findings parallel those previously noted by Kuwabara in rabbit...
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Retinas subjected to the damaging effects of mechanical trauma or intravitreal blood injection, and emphasize the nonspecific nature of these reparative changes.

The pigment epithelial cells still showed no evidence of migration or proliferation. The microvilli at the apical borders probably represented interdigitations of the pigment epithelium with the cytoplasmic processes of the Müller cells. The inner retinal layers, Bruch's membrane, and the choriocapillaris remained intact.

**Pigmented rabbits.** Because the previously reported investigations of oxygen-induced visual cell degeneration utilized only albino rabbits, it was considered important to test whether the albino state rendered the retina more susceptible to oxygen toxicity. Melanin pigment is thought to be a good acceptor of free radicals and it has been postulated that melanin serves in this fashion as a naturally occurring antioxidant. Therefore, pigmented adult rabbits were exposed to 100 per cent oxygen under the same conditions as described for the albino rabbits. A degeneration of retinal visual cells identical in nature and severity to that produced in the albino animal was demonstrated with light and electron microscopy.

**DL-alpha-tocopherol acetate supplementation.** Rather than exerting a protective effect on the retina, systemically administered DL-alpha-tocopherol acetate either afforded no protection or actually appeared to enhance the deleterious effects of oxygen on the visual cells of the treated as compared with the control animals.

**Neoprene casts of the choroid.** No alterations in the structure or patency of the choroidal vessels were seen in the casts from the oxygen-treated animals. Consistent in all specimens, both normal and experimental, was the concentration of large and small arteries in the posterior region of the choroid, especially that portion below the optic disc which supplies the area centralis of the retina. The proximity of this dense arterial network to the main trunks of the short posterior ciliary arteries suggests that highly oxygenated blood under a greater perfusion pressure is distributed to the choriocapillaris supplying the area centralis than is distributed to the more peripheral regions. This contention is supported by the demonstration in the present study of a low glycogen content in the Müller cells of the area centralis of the normal rabbit. Kuwabara and Cogan have shown that the glycogen content of the Müller cells is reciprocally related to the degree of vascularity and presumed oxygenation of the retina. With the exception of the area centralis the Müller cells of the essentially avascular rabbit retina have a high glycogen content. The paucity of glycogen in the area centralis suggests again that this region is better oxygenated. Since the damaging effect of oxygen seems to be directly related to the concentration of oxygen achieved in the exposed tissues, this would help to explain the predisposition of the area centralis to oxygen toxicity. It remains to be shown whether other metabolic differences characterize this region.

**Discussion**

The earliest recognizable electron microscopic changes in oxygen-induced visual cell degeneration were found within the intracellular membrane-bound organelles of the photoreceptor inner segment and nucleus. The occurrence of swelling in these structures suggests that excessive oxygen or a substance related to its metabolism produced a functional deficiency in the lipoprotein membranes constituting these intracellular elements. Similar cytoplasmic vesicles in the inner segment of the visual cells have been described by Lasansky and deRobertis as an early structural feature of iodoacetate poisoning of the rabbit retina.

Further work is necessary to determine whether these early changes seen by electron microscopy are reversible. Noell has demonstrated a depression of elements of the ERG induced by oxygen in rabbits which he found to be reversible in the
early stages. In order to produce permanent ERG changes, animals had to be exposed to oxygen for several hours after the initial ERG depression. It would be interesting to correlate the early reversible ERG changes with the early structural changes found by electron microscopy. It is possible that both the ERG depression and the swelling of the intracellular elements reflect a functional breakdown of lipoprotein membranes.

Following the early ultrastructural changes there was a rapid degeneration of the entire visual cell indicating an irreversible insult to vital cell processes. Since the pigment epithelium, choriocapillaris, and Bruch's membrane remained essentially normal in the early stages, it appears as though the photoreceptor outer segment degeneration was secondary to injury of the more proximal portion of the visual cell. This contrasts with the ultrastructural alterations produced by certain other retinal receptor toxins such as sodium iodate, where the initial lesion has been demonstrated in the pigment epithelium, with subsequent degeneration of the photoreceptor outer segments. 13

Noell4 in his light microscopical study concluded that certain visual cells of the rabbit survived prolonged exposure to high oxygen concentrations. He identified the resistant cells as cones by their large nuclei and fine distribution of nuclear chromatin. In the present study the surviving visual cell nuclei did not seem to show any distinguishing structural features. It is true that some of the nuclei found in the outer nuclear layer after prolonged exposure to oxygen were large and contained finely dispersed nuclear chromatin. However, electron microscopy confirmed that these were the nuclei of Müller cells which had migrated into the outer nuclear layer.

There has been considerable lack of agreement concerning the ultrastructural identification of "rods" and "cones" in the normal rabbit retina.6, 14 This makes it difficult to evaluate the relative susceptibility of "rods" and "cones" to oxygen toxicity. Various authors have attempted to distinguish between normal "rods" and normal "cones" on the basis of the contrasting structural features of either the inner and outer segments or the nuclei or the synapses of the visual cells. In the oxygen-poisoned retina the inner and outer segments of the surviving visual cells, especially in the area centralis, were too distorted to allow detailed study for identification. However, examination of the visual cell synapses by electron microscopy demonstrated a preponderance of complex beta-type synaptic pedicles among the surviving cells within the degenerated area centralis. Therefore, these surviving cells, whether they be designated "rods" or "cones," were morphologically distinctive and physiologically distinctive as evidenced by their relative resistance to oxygen poisoning.

It was disappointing that the intraperitoneal administration of alpha-tocopherol acetate failed to show a protective effect against oxygen-induced visual cell degeneration. The peroxidation of the lipids in cell membranes under conditions of elevated oxygen concentrations has been suggested as a possible mechanism of oxygen toxicity.15 Alpha-tocopherol acetate is capable of inhibiting the peroxidation of microsomal and mitochondrial lipids in vitro.16 In addition, several workers17, 18 have succeeded in minimizing oxygen-induced neurological, hematological, and pulmonary damage in animals by the supplementation of a laboratory diet with alpha-tocopherol acetate. It is possible that the occurrence of more severe visual cell degeneration in some of the alpha-tocopherol acetate supplemented animals in the present study was due to a relative protection against pulmonary and hematological damage, allowing a greater oxygen saturation of the blood and delivery of a higher dose of oxygen to the retina.

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