

Ascorbic Acid Content of Human Corneal Epithelium

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PURPOSE. To measure the concentration of ascorbic acid in the human corneal epithelium.

METHODS. Corneal epithelium was removed from postmortem eyes 4 to 16 hours after death and ascorbate measured by high-performance liquid chromatography.

RESULTS. The concentration of ascorbate was 1.33 ± 0.48 mg/gm wet weight (mean \pm SD), estimated to be 14 times its concentration in the aqueous humor.

CONCLUSIONS. Ascorbate can protect the basal layer of the epithelium by absorption of incident ultraviolet radiation. (*Invest Ophthalmol Vis Sci.* 2000;41:1681-1683)

The corneal epithelium of animals has been known to contain very high concentrations of ascorbic acid, first reported by Pirie¹ in rabbits and oxen. Pirie's observations were consistent with the notion that ascorbate enters the aqueous humor, diffuses through the endothelium into the stroma, and then is concentrated by the epithelial cells. This idea was supported by the work of Reim et al.² who found the concentration of ascorbate in the epithelium of rabbits to be as high as 2 mg/gm wet weight, 8 times the concentration in the aqueous humor.

The dynamics of ascorbate in the anterior segment have been the subject of investigation since the discovery by Birch and Dann³ of the high concentration in the aqueous humor. Mueller and Buschke⁴ and, later, Kinsey⁵ showed that the aqueous humor concentrations were increased even more after parenteral administration of ascorbic acid, and Reim et al.² demonstrated the same effect in the corneal epithelium. Kinsey showed it was the reduced form rather than the oxidized form that was transported.⁶ A number of authors have since determined that an active transport system demonstrating saturable kinetics is present in the ciliary body and moves ascorbic acid from the plasma into the posterior chamber.⁷⁻¹⁹ Excellent reviews of ascorbic acid metabolism in the eye have been published by Rose and Bode²⁰ and by Delamere.²¹

Recently, two specific ascorbate transport proteins have been discovered and sequenced.²² One of these proteins, termed by the authors "sodium-dependent vitamin C transporter 2," is expressed both in the ciliary epithelium and in the corneal epithelium of the rabbit eye. The identification of this transporter confirms what had been the putative mechanism responsible for the high concentrations of ascorbic acid in the eye. The histologic localization of this transporter suggests that the very high concentration of ascorbic acid in the corneal epithelium results from a two-stage transfer process, first from

the plasma to the aqueous humor and second from the aqueous humor to the corneal epithelium. This was Pirie's original hypothesis.

Recently, Ringvold et al.²³ measured ascorbate in the corneal epithelium of different animals and found the highest concentrations in diurnal species that encounter the highest environmental levels of ultraviolet radiation. Ringvold et al.^{24,25} emphasized that ascorbic acid is an excellent absorber of UV radiation between 280 and 310 nm and that it has an absorption curve that roughly matches the absorption curves of protein and nucleic acids in this region of the spectrum. Pitts and Tredici²⁶ show that the absorption spectrum of ascorbic acid is the inverted action spectrum of UV damage to the cornea. These findings, coupled with the finding that diurnal animals have the highest concentrations of ascorbic acid in the anterior chamber,^{27,28} suggest that the eye is able, either through evolution or physiological adaptation, to create its own "sunscreen" to protect itself from the deleterious effects of ambient UV radiation.

Humans, like diurnal mammals, have a high concentration of ascorbate in the aqueous humor, but the concentration of ascorbic acid in the corneal epithelium of humans has not been reported. The purpose of this article is to report that human corneal epithelium contains very high concentrations of ascorbic acid and to speculate on its role in the health of the cornea.

METHODS

Eighteen human eyes were obtained at autopsy of 9 subjects and transferred to a laboratory for processing. The lapse of time between death and collection of epithelium and aqueous humor was recorded, and in most instances was less than 10 hours. Corneal epithelium was scraped off with a glass knife and placed in preweighed 1.5-ml microcentrifuge tubes. The tubes were reweighed to determine the weight of the epithelial specimen. Cooled meta-phosphoric acid, 200 μ l, was then added to the tube. The epithelial specimen was then homogenized in its tube with a matching pestle. Aqueous humor was drained from the anterior chamber and diluted 1:1 with cooled 10% meta-phosphoric acid in a 1.5-ml microcentrifuge tube. Both tubes (epithelium and aqueous humor) were then stored at -70°C .

Within 10 days the specimens were warmed to room temperature, and the epithelial specimen was centrifuged at 12,000g for 10 minutes. Twenty-microliter aliquots were in-

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Supported in part by Grants EY 00634 (RFB) and EY 02037 (WMB) from the National Institutes of Health (Bethesda, Maryland); by Research to Prevent Blindness (New York, New York); and by the Mayo Foundation (Rochester, Minnesota).

Submitted for publication September 27, 1999; revised January 11, 2000; accepted January 18, 2000.

Commercial relationships policy: N.

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TABLE 1. Data from Measurement of Ascorbate in Epithelium and Aqueous Humor

Eye	Age/Sex	Corneal Epithelium, mg/gm	Aqueous Humor, mg/ml	Ratio* C/A	Elapsed Time†, hours
Subject 1	80/F				4
OD		2.30	0.23	14.4	
OS		2.50	0.19	18.6	
Subject 2	82/F				6.5
OD		1.00	0.09	15.1	
OS		1.04	0.12	12.2	
Subject 3	83/M				16
OD		1.61	0.12	19.0	
OS		(0.46)	—	—	
Subject 4	30/F				6
OD		1.04	0.04	40.1	
OS		0.86	0.04	32.3	
Subject 5	91/F				6.7
OD		1.50	0.33	6.4	
OS		1.66	0.30	8.0	
Subject 6	46/F				4
OD		(1.11)	—	—	
OS		1.21	0.18	9.7	
Subject 7	79/M				9
OD		1.27	0.20	9.1	
OS		0.97	0.18	7.7	
Subject 8	44/M				10
OD		0.87	0.36	3.4	
OS		1.10	0.35	9.4	
Subject 9	50/F				12
OD		1.28	0.25	7.3	
OS		1.06	0.17	8.9	
Mean ± SD		1.33 ± 0.48	0.20 ± 0.10	13.6 ± 10.0	

Data in parentheses were measured, but not included in mean.

* Water content of epithelial cells assumed to be 70% of wet weight.

† Time between death of patient and processing of eye.

jected into the high-performance liquid chromatography (HPLC) system. All specimens were measured in triplicate and compared with standards of 0.01, 0.1, 0.25, and 0.5 mg/ml prepared from analytical grade ascorbic acid dissolved in 10% meta-phosphoric acid.

A Beckman Gold HPLC system with a photodiode array detector was used. A Supelcosil LC-18, 250 × 4.6 mm, 5- μ m particle size column was used with a guard column containing the same material. The mobile phase was HPLC grade water acidified to a pH of 2.2 with sulfuric acid. The flow was set to 1.0 ml/min. The wavelength of the detector was set to 243 nm.

The study was approved by the Institutional Review Board of Mayo Clinic.

RESULTS

In two eyes, adequate aqueous humor samples could not be obtained. In 16 eyes, successful measurements of epithelial and aqueous humor ascorbate were made (Table 1). The concentration of ascorbate in the corneal epithelium of these 16 eyes was 1.33 ± 0.48 mg/gm wet weight (mean \pm SD) and in the aqueous humor was 0.20 ± 0.10 mg/ml. If the water content of the corneal epithelium is assumed to be 70% of the wet weight, the ratio of ascorbate in the epithelium to that in the aqueous humor was 13.6 ± 10 .

DISCUSSION

If the ascorbate were uniformly distributed in epithelial water, its concentration would be 1.9 mg/ml of cell water, nearly 14

times higher than its concentration in the aqueous humor. If the concentration in the aqueous humor were 20 times that in plasma,²¹ the overall concentration gradient between the epithelium and the plasma can be nearly 300:1.

Humans lack the enzyme L-gulonolactone oxidase, rendering them unable to carry out the final step in the synthesis of ascorbic acid.²⁹ Consequently, the very high concentration in the corneal epithelium cannot be explained by synthesis in situ. Instead, the enormous concentration gradient between the plasma and the eye must be created by the action of the recently discovered transporter protein, sodium-dependent transporter protein 2, that is present in the ciliary epithelium and in the corneal epithelium.²²

The concentration of ascorbate in the corneal epithelium is the highest of any known tissue concentration in the eye and higher than any other reported tissue in the body. Its molarity, 11 mM/l, approaches that of many key components of cytosol. At this concentration, for example, ascorbic acid is 50 times more concentrated than oxygen in water in equilibrium with atmospheric air at room temperature. Ascorbic acid alone would account for approximately 4% of the molarity of all the solute molecules in the cytosol!

The age of our subjects, their terminal condition, and the delay between death and tissue processing all favor the idea that the concentrations of ascorbic acid reported here are lower than those found in healthy, living humans. At such high concentrations, ascorbic acid could serve to protect the deeper layers of the cornea from radiation damage, such as the basal epithelial layer, the stromal keratocytes, and the corneal endothelium. Ascorbic acid could carry out an energy-absorbing

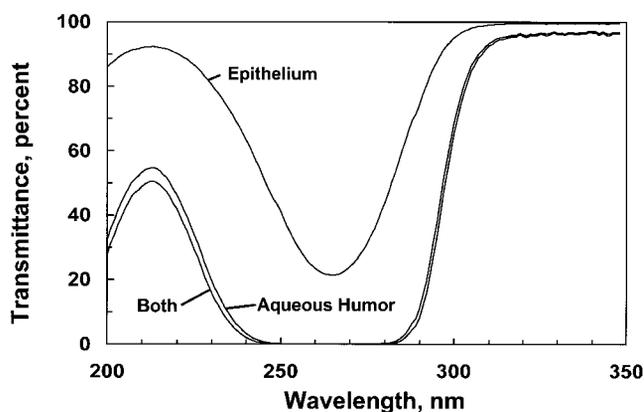


FIGURE 1. Calculated transmittance of a 50- μ m-thick layer of fluid containing 1.6 mg/ml ascorbate, simulating the epithelial layer of the cornea, and a 3-mm-thick layer of fluid containing 0.2 mg/ml ascorbate, simulating the combined stromal layer of the cornea and aqueous humor of the anterior chamber. The concentration in milligrams of ascorbate per milliliter cell volume has been calculated to be 1.6 based on an assumed density of corneal epithelium of 1.2 g/ml.

function for the central area of the cornea, a function that can be carried out by melanin pigment in the interpalpebral region of the limbus, an area that is often pigmented, especially in darker races.

It is not clear what role ascorbic acid might play in protecting the cornea from radiation. However, if ascorbate is evenly distributed throughout the corneal epithelium, ascorbate alone would absorb 77% of the incident radiation at wavelengths likely to be most dangerous to the genetic material of the basal layer. Ascorbate could also protect the epithelium of the lens. Before reaching the lens, 99.96% of radiation at 260 nm would have been absorbed by ascorbate in the intervening structures. These absorbances are derived from the expected transmittance of a layer of fluid 50- μ m-thick containing ascorbate at the concentration found in epithelium and the expected transmittance of a layer of fluid 3-mm-thick containing ascorbate at the concentration found in the aqueous humor (see Figure).

Ocular ascorbate would rate as having a Sun Protective Factor (SPF) of 4 (at this wavelength) for the basal layer of the cornea and 2500 for the lens epithelial layer. Thus, Ringvold's hypothesis about ascorbate's role in the eye as an absorber of ultraviolet radiation is certainly correct.^{24,25}

References

- Pirie A. Ascorbic acid content of cornea. *Biochem J.* 1946;40:96-100.
- Reim M, Seidl M, Brucker K. Accumulation of ascorbic acid in the corneal epithelium. *Ophthalmic Res.* 1978;10:135-139.
- Birch TW, Dann WJ. Estimation and distribution of ascorbic acid (vitamin C) and glutathione in animal tissues. *Nature.* 1933;131:469-470.
- Mueller HK, Buschke W. Vitamin C in Linse, Kammerwasser und Blut bei normalem und pathologischem Linsentoffwechsel. *Graefes Arch Ophthalmol.* 1934;108:368-390.
- Kinsey VE. Transfer of ascorbic acid and related compounds across the blood aqueous barrier. *Am J Ophthalmol.* 1947;30:1262-1266.
- Kinsey VE. Dehydroascorbic acid—ascorbic acid in the aqueous humor of rabbits. *Am J Ophthalmol.* 1950;33:257-268.

- Linnér E. Ascorbic acid as a test substance for measuring relative changes in the rate of plasma flow through the ciliary processes, II: the effect of unilateral ligation of the common carotid artery on the blood flow through the uveal tract as measured directly in a vortex vein. *Acta Physiol Scand.* 1952;26:70-78.
- Linnér E. Ascorbic acid as a test substance for measuring relative changes in the rate of plasma flow through the ciliary processes, I: the effect of unilateral ligation of the common carotid artery in rabbits on the ascorbic acid content of the aqueous humour at varying plasma levels. *Acta Physiol Scandinav.* 1952;26:57-69.
- Becker B, Linnér E. Ascorbic acid as a test substance for measuring relative changes in the rate of plasma flow through the ciliary processes, III: the effect of pre-ganglionic section of the cervical sympathetic in rabbits on the ascorbic acid content of the aqueous humour at varying plasma levels. *Acta Physiol Scand.* 1952;26:79-85.
- Kinsey VE. Comparative chemistry of aqueous humor in posterior and anterior chambers of rabbit eye: its physiologic significance. *Arch Ophthalmol.* 1953;50:401-417.
- Langham ME. The use of ascorbic acid to measure the rate of flow of plasma through the ciliary processes. *J Physiol.* 1955;130:1-8.
- Linnér E, Nordström K. Transfer of D-isoascorbic acid and L-ascorbic acid into guinea pig eyes. In: von Bahr G, Bietti GB, Ten Doesschate J, et al., eds. *Documenta Ophthalmologica Advances in Ophthalmology.* The Hague: Dr. W Junk NV; 1969:164-170.
- Becker B. Ascorbate transport in guinea pig eyes. *Invest Ophthalmol.* 1967;6:410-415.
- Kodama T, Kabasawa I, Tamura O, Reddy VN. Dynamics of ascorbate in the aqueous humor and tissues surrounding ocular chambers. *Ophthalmic Res.* 1985;17:331-337.
- Delamere NA, Williams RN. A comparative study on the uptake of ascorbic acid by the iris-ciliary body of the rabbit, guinea pig and rat. *Comp Biochem Physiol.* 1987;88B:847-849.
- Chu T-C, Candia OA. Active transport of ascorbate across the isolated rabbit ciliary epithelium. *Invest Ophthalmol Vis Sci.* 1988; 29:594-599.
- DiMattio J. A comparative study of ascorbic acid entry into aqueous and vitreous humors of the rat and guinea pig. *Invest Ophthalmol Vis Sci.* 1989;30:2320-2331.
- Bode AM, Vanderpool SS, Carlson EC, Meyer DA, Rose RC. Ascorbic acid uptake and metabolism by corneal endothelium. *Invest Ophthalmol Vis Sci.* 1991;32:2266-2271.
- DiMattio J. Ascorbic acid entry into cornea of rat and guinea pig. *Cornea.* 1992;11:53-65.
- Rose RC, Bode AM. Ocular ascorbate transport and metabolism. *Comp Biochem Physiol.* 1991;100A:273-285.
- Delamere NA. Ascorbic acid and the eye. *Subcell Biochem.* 1996; 25:313-329.
- Tsukaguchi H, Tokui T, Mackenzie B, et al. A family of mammalian Na⁺-dependent L-ascorbic acid transporters. *Nature.* 1999;399: 70-75.
- Ringvold A, Anderssen E, Kjønneksen I. Ascorbate in the corneal epithelium of diurnal and nocturnal species. *Invest Ophthalmol Vis Sci.* 1998;39:2774-2777.
- Ringvold A. Cornea and ultraviolet radiation. *Acta Ophthalmol.* 1980;58:63-68.
- Ringvold A. The significance of ascorbate in the aqueous humour protection against UV-A and UV-B. *Exp Eye Res.* 1996;62:261-264.
- Pitts DG, Tredici TJ. The effects of ultraviolet on the eye. *Am Ind Hyg Assoc J.* 1971;32:235-246.
- Reiss GR, Werness PG, Zollman PE, Brubaker RF. Ascorbic acid levels in the aqueous humor of nocturnal and diurnal mammals. *Arch Ophthalmol.* 1986;104:753-755.
- Koskela TK, Reiss GR, Brubaker RF, Ellefson RD. Is the high concentration of ascorbic acid in the eye an adaptation to intense solar irradiation? *Invest Ophthalmol Vis Sci.* 1989;30:2265-2267.
- Sato P, Udenfriend S. Studies on ascorbic acid related to the genetic basis of scurvy. *Vitam Horm.* 1978;36:33-52.