
MINIREVIEW

UV Rays, the Prooxidant/Antioxidant Imbalance in the Cornea and Oxidative Eye Damage

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Summary

In this minireview, the factors involved in the development of corneal injury due to an increased amount of UVB rays are summarized. Experimental studies have shown that an increased number of UVB rays leads to a profound decrease in corneal antioxidants (high molecular weight, antioxidant enzymes as well as low molecular weight, mainly ascorbic acid) so that a prooxidant/antioxidant imbalance appears. The decrease of corneal antioxidant protective mechanisms results in oxidative injury of the cornea and causes damage of the inner parts of the eye by UVB rays and by reactive oxygen species generated by them.

Key words

Cornea • UV rays • Prooxidant/antioxidant imbalance

Introduction

Recently, an increased danger for the eye from UV rays has been linked to the decrease in stratospheric ozone. The health risks associated with ozone depletion are caused by the enhanced UVA radiation in the environment and increased penetration of UV radiation of shorter wavelength (between 280 nm and 320 nm, UVB rays) (Zigman 1993, Tenkate 1999, Ma *et al.* 2001, Kabuyama *et al.* 2002). The anterior eye segment damage from UV exposure includes effects on the cornea, lens, iris, and associated epithelial and conjunctival tissues

(Reddy *et al.* 1998, Merriam *et al.* 2000, Čejková *et al.* 2000, 2001, Midelfart *et al.* 2002). The most common acute effect of environmental ultraviolet radiation on the cornea is photokeratitis. Chronic eye conditions likely to increase with ozone depletion include cataract, squamous cell carcinoma, ocular melanoma, and a variety of corneal and conjunctival pathologies, e.g. pterygium and pinguecula (Ringvold 1980, Longstreth *et al.* 1998, Ringvold 1998, Hockwin *et al.* 1999, West 1999, Balasubramanian 2000, Berwick 2000, De Gruil 2000).

One of the causes of ocular damage induced by UV irradiation is the generation of reactive oxygen

species (Riley 1988). Reactive oxygen species (hydrogen peroxide, singlet oxygen and oxygen free radicals such as superoxide anions and hydroxyl radicals) are a danger for biological systems. They might cause cellular damage by reacting with lipids, proteins and DNA (Chace *et al.* 1991, Kehrer 1993, Mehlhase and Grune 2002). A number of pathologies have been attributed to the action of reactive oxygen species, and one of the dominant theories of aging suggests that senescent changes are a consequence of the accumulated action of these toxic products (Emerit 1992, Stadtman 2001, Hensley and Floyd 2002). According to Ogura *et al.* (1991), reactive oxygen species are important mediators of lipid peroxidation in the epidermis exposed to UV light. Reactive oxygen species are suggested to play a major role in a number of specific pathological conditions of intraocular tissues, such as cataract formation and retinal degeneration (Mittag 1984). In the cornea, reactive oxygen species might cause the direct cleavage of stromal glycosaminoglycans and alteration of their physiological properties. Stromal glycosaminoglycans become more susceptible to degradation by tissue enzymes and enzymes released from stimulated phagocytic cells (Carubelli *et al.* 1990).

The antioxidant enzymes in the cornea

Ocular tissues and fluids contain both low molecular weight antioxidants (such as ascorbic acid, glutathione and alpha-tocopherol) and high molecular weight antioxidants (such as catalase, superoxide dismutase, glutathione peroxidase and reductase) that play a key role in protecting against oxidative damage. Superoxide dismutase (SOD: EC.1.15.1.1.) catalyses dismutation of superoxide to peroxide and molecular oxygen. Thus, this enzyme protects the ocular tissues from the superoxide radicals. At least three different forms of superoxide dismutase have been described in mammalian tissues: cytosolic copper-zinc superoxide dismutase, mitochondrial superoxide dismutase (Weisiger and Fridovich 1973) and the high molecular weight extracellular superoxide dismutase (Marklund 1982). This enzyme has been identified in the normal rabbit corneal epithelium (and also corneal endothelium, lens, iris, ciliary body and retina) by Bhuyan and Bhuyan (1978). Redmond *et al.* (1984) found superoxide dismutase (identical to the Cu-Zn superoxide dismutase) using biochemical as well as immunohistochemical methods in the corneal epithelium and endothelium of

rats, dogs, rabbits and humans. The enzyme quantities detected by radioimmunoassay and bioactivity were similar, providing evidence that the enzyme is present in a biologically functional form. Immunohistochemical techniques demonstrated an association of the enzyme with the cytoplasm of these layers. In rats, besides the corneal epithelium and endothelium, Rao *et al.* (1985) also detected superoxide dismutase in other eye structures – the apical regions of the posterior epithelium of the iris, the nonpigmented inner ciliary epithelium, the lens epithelium, the inner segments of the photoreceptor cell layer of the retina, and the retinal pigment epithelium – using immunohistochemical techniques. Behndig *et al.* (1998) described superoxide dismutase isoenzymes in the human eye; in the cornea, Cu/Zn superoxide dismutase and extracellular superoxide dismutase were also found. Frederiks and Bosch (1997) developed a method for the detection of superoxide dismutase activity *in situ*. This technique was employed by Čejková *et al.* (2000) for the detection of superoxide dismutase activity in the cornea of rabbits. Glutathione peroxidase (GSH : H₂O₂ oxidoreductase, EC 1.11.1.6.) is a very important enzyme scavenging hydrogen peroxide. Glutathione peroxidase was detected immunohistochemically by Atalla *et al.* (1988, 1990) in rat corneal epithelium (and the corneal endothelium, the choroid, the inner segment of the photoreceptors and the retinal pigment epithelium). Biochemically, glutathione peroxidase activity has been described in various eye tissues of rabbits (including the corneal epithelium) (Bhuyan and Bhuyan 1977). Catalase (H₂O₂ : H₂O₂ oxidoreductase, EC 1.11.1.9) protects ocular tissues against hydrogen peroxide and also protects superoxide dismutase from inactivation by hydrogen peroxide. Catalase was investigated biochemically in the normal rabbit corneal epithelium (also the corneal endothelium, the lens epithelium, the ciliary body and the retina) by Bhuyan and Bhuyan (1970) and immunohistochemically by Atalla *et al.* (1987). Ocular catalase was also studied by Mayer (1980) using Warburg's respirometer, and the results were given in microliters of O₂ per mg of soluble protein. Catalase activity within the normal corneal epithelium and endothelium was detected in rabbits by Čejková and Lojda (1994) using a modified method of Novikoff and Goldfischer as given by Lojda *et al.* (1979) for peroxisomes. Similar results were also obtained by the method of Angermüller and Fahimi (1981) modified by Frederiks and Bosch (1995).

Under physiological conditions, these antioxidant enzymes protect the cornea against oxidative stress (Bhuyan and Bhuyan 1977, 1978, Redmond *et al.* 1984, Atalla *et al.* 1987, 1988, 1990, Čejková and Lojda 1996, Behndig *et al.* 1998, Čejková *et al.* 2000). The danger to the eye appears to stem from an increased amount of UV (UVA + UVB) radiation. The cornea absorbs 92 % of UVB and 60 % of UVA radiation and is most sensitive to UVB damage (Zigman 1993). The aqueous humor, containing ascorbic acid, proteins and some aminoacids (tyrosine, phenylalanine, cysteine, tryptophane), is also responsible for UVB absorption so that only a small number of UV rays reaches the intraocular lens (Ringvold 1998). The lens acts to filter light between 300-400 nm from reaching the retina (Eaton 1994-1995).

Mitchell and Cenedella (1995) examined the quantitative contribution of major chemical fractions of the whole bovine cornea to UV absorption between 240 and 300 nm. The cornea was divided into water-insoluble, non-protein small water-soluble, water-soluble protein, and lipid-soluble fractions. The absorbance by individual fractions was dependent on the wavelength. The insoluble fraction (largely collagen) absorbed 50 % of UV between 240 and 280 nm, whereas in the range of 290-300 nm, the water-soluble plus lipid-soluble fractions absorbed 60 % of the total absorption. Kolozsvari *et al.* (2002) determined UV absorbance of the corneal layers (epithelium, Bowman layer, stroma). These authors found that the anterior part of the cornea (the epithelium and Bowman layer) was much more important for the absorption than the posterior layers of the cornea.

Ringvold (1996, 1998) studied UV absorption and UV-induced fluorescence in the cornea and aqueous humor. Three different mechanisms in UVB protection were described: the absorption of UV below 310 nm, fluorescence-mediated ray transformation to longer wavelength and fluorescence reduction. In the cornea, epithelium had the key role (Ringvold 1997). Ringvold *et al.* (2000) analyzed the distribution of ascorbate in the anterior bovine eye. The highest ascorbate concentration was found in the corneal epithelium, with significantly higher values in the central (1.56 mg/g) than in the peripheral (1.39 mg/g) region. The ascorbate content was similar in the corneal stroma (0.22 mg/g), Descemet's membrane/endothelium (0.22 mg/g) and the aqueous humor (0.21 mg/ml). The sclera and the conjunctiva showed lower values. The authors found the peak

ascorbate concentration in the central corneal epithelium covering the pupillary area. The authors suggested that the high ascorbate concentration in this area was compatible with the idea that the ascorbate might act as an UV filter shielding the internal structures from radiation damage.

It follows from the above mentioned studies that both high molecular weight antioxidants (antioxidant enzymes) and also low molecular weight antioxidants (mainly ascorbic acid) together with tissue components absorb and detoxify UVB radiation and the reactive oxygen species generated by them (for review see Rose *et al.* 1998).

Experimental studies showed that UVB rays (and the reactive oxygen species generated by them) cause morphologic disturbances in the cornea. Already a single irradiation of the cornea with UVB rays was sufficient to block the proliferation of epithelial cells (Haaskjold *et al.* 1993). Higher doses of UVB rays resulted in a considerable reduction in epithelial thickness (Koliopoulos and Margaritis 1979). Using slit-lamp biomicroscopy as well as light and electron microscopy Cullen (1980) described a loss of the superficial corneal epithelial layers and selective UV-induced autolysis of wing-shaped cells (cells of the middle layer of the corneal epithelium). Along with morphological disturbances, a decrease of both UV absorption and removal of reactive oxygen species by cornea and aqueous humor appeared. It was shown that this decrease was closely dependent on UV wavelength, dose and frequency of irradiation (single or repeated irradiation). Čejková *et al.* (2000) reported that UVB rays (not UVA rays, see Fig. 1) caused a decrease of antioxidant enzymes (mainly catalase and glutathione peroxidase) in the corneal epithelium of rabbits already after 4 days of repeated irradiation. Löfgren and Söderberg (2001) found a decrease in lactate dehydrogenase in the corneal epithelium after irradiation of the rat eye with UVB rays. It should be mentioned that in rabbits, UVB rays evoked a decrease in Na⁺-K⁺-dependent ATPase first in the corneal epithelium and later in the corneal endothelium, which was accompanied by increased corneal hydration and changes in corneal transparency (Čejková and Lojda 1996). Na⁺-K⁺-dependent ATPase is the major system of transcorneal sodium and chloride transport, and, hence, a major controller of corneal hydration (Redmond *et al.* 1984). For the maintenance of normal corneal hydration and transparency, the corneal endothelium plays the key role. Under physiological conditions, the corneal endothelium

is exposed to the oxidants of the aqueous humor. Hydrogen peroxide is present in normal rabbit aqueous humor in a 20 μM concentration (Ringvold 1980). The corneal endothelium contains antioxidants (Bhuyan and Bhuyan 1984, Redmond *et al.* 1984), and it is therefore able to adequately defend against a physiological level of hydrogen peroxide. However, the corneal endothelium is very susceptible to damage by increased levels of hydrogen peroxide (Riley and Giblin 1983, Riley *et al.* 1987, Hull and Green 1989, Ren and Wilson 1994). This might lead to increased corneal hydration and corneal opacity.

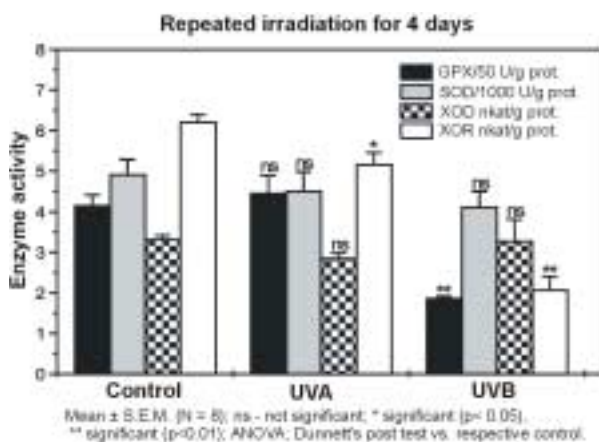


Fig. 1. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), xanthine oxidase (XOD) and xanthine oxidoreductase (XOR) in the scraped epithelium of a rabbit cornea repeatedly irradiated with UVA or UVB rays for 4 days. The open eyes were irradiated with UVA lamp or UVB lamp (Illkirch Cedex, France; 312 or 365 nm wavelength, 6 W) from a distance of 0.03 m. Only corneas were irradiated; the rest of the eye surface was protected from UV rays. The animals were irradiated for 5 min once daily for 4 days. (For more details on experimental conditions and biochemical determination of enzyme activities, see Čejková *et al.* 2000 and 2001). Of the enzymes studied, UVB rays significantly decreased the activities of GPX and XOR; UVA rays decreased only the activity of XOR.

The increase of hydrogen peroxide (and the decrease in antioxidants) in the aqueous humor as a possible risk factor in cataractogenesis has been extensively studied after argone fluoride 193 nm excimer laser corneal surgery. Costaglia *et al.* (1994) found in albino rabbits that after photorefractive keratectomy hydrogen peroxide and oxidized glutathione increased in the aqueous humor, whereas the ascorbic acid and reduced glutathione concentration decreased. Moreover, all these variations significantly correlated with the cumulative UV dose used. Bilgihan *et al.* (1996)

described that corneal photoablation with argone fluoride 193 nm excimer laser decreased superoxide dismutase levels in the aqueous humor.

Prooxidant enzymes in the cornea (H_2O_2 -producing oxidases)

There exist six H_2O_2 -producing oxidases: monoamine oxidase, benzylamine oxidase, histamine oxidase, alpha hydroxy acid oxidase, D-amino acid oxidase and xanthine oxidase. The role of oxidases in the cornea and anterior eye surface has been studied (monoamine oxidase – Neufeld *et al.* 1982, Klyce *et al.* 1982, Kalminia *et al.* 1977; diamine oxidase and benzylamine oxidase – Ziche *et al.* 1987; D-amino acid oxidase – Čejková *et al.* 2001; alpha hydroxy acid oxidase – Zeng *et al.* 1995, Čejková *et al.* 2001).

Of the oxidases that produce reactive oxygen species, the greatest attention has been devoted to xanthine oxidase. Xanthine oxidoreductase is involved in the degradation of adenosintriphosphate to urate by converting hypoxanthine *via* xanthine into uric acid. This enzyme exists in two forms: xanthine oxidase (xanthine: O_2 , EC 1.2.3.2), an oxygen-reducing form generating superoxide anions and hydrogen peroxide, and xanthine dehydrogenase (xanthine: NAD^+ , EC 1.1.1.204), a NAD^+ -reducing form. Kooij *et al.* (1992) and Frederiks and Bosch (1996) proposed that the main function of this enzyme is not the production of superoxide radicals and/or hydrogen peroxide, but rather the metabolism of xanthine to uric acid, which can act as a potent antioxidant. This suggestion is based on the finding of enzyme activity in epithelial as well as endothelial cells of various organs that are subject to relatively high oxidant stress; therefore, the authors postulated that in these cells, xanthine oxidoreductase is involved in the antioxidant enzyme defense system. Under various (patho)physiological conditions, xanthine dehydrogenase can be reversibly converted into xanthine oxidase (e.g. Granger *et al.* 1986) *via* oxidation of sulphhydryl groups and irreversibly by proteolysis (Kooij *et al.* 1992). Kooij *et al.* (1994) demonstrated biochemically that the conversion of xanthine dehydrogenase to xanthine oxidase occurs exclusively *in vitro* or extracellularly. The physiological role of xanthine oxidase is still rather unclear (Kooij *et al.* 1994). Some authors have proposed a bactericidal function for the enzyme (Jarasch *et al.* 1981, Tubaro *et al.* 1980a,b, van den Munckhof 1996, Gossrau *et al.* 1990, Čejková *et al.* 2001).

Under pathological conditions, a role of xanthine oxidase in hypoxia-reoxygenation injury has often been proposed (Kooij *et al.* 1990, 1994) and critically reviewed (Saugstad 1996, Li and Jackson 2002). As mentioned above, Kooij *et al.* (1994) described in the liver that the conversion of xanthine dehydrogenase to xanthine oxidase might occur exclusively *in vitro* or extracellularly. In contrast, Cote *et al.* (1996) found in rat lung that the exposure to hypoxia produced a significant increase in lung tissue xanthine oxidase activity and an increase in the ratio of xanthine oxidase to xanthine dehydrogenase. Histochemically, the role of xanthine oxidase in the corneal damage evoked by prolonged wearing of hydrophilic contact lenses, long-lasting corneal hypoxia and rapid reoxygenation of the cornea after contact lens removal was described by Čejková *et al.* (1998). It was suggested that xanthine oxidoreductase was released from the corneal epithelium into tears, converted to xanthine oxidase and caused additional damage to the superficial layers of the corneal epithelium by reactive oxygen species.

Corneal prooxidant/antioxidant enzymatic imbalance evoked by UVB rays

High level of H₂O₂-producing oxidase activities in the cornea after UVB irradiation

Čejková *et al.* (2001) found that early irradiation of the cornea with UVB rays evoked a gradual increase in xanthine oxidase and D-amino acid oxidase activities in the corneal epithelium and endothelium. Inflammatory cells (mainly polymorphonuclear leukocytes) present in the corneal stroma and later also in the anterior chamber showed high activities of both enzymes. Only after a longer irradiation procedure, xanthine oxidase and D-amino acid oxidase decreased in the thinned corneal epithelium, which paralleled morphological disturbances. The superficial epithelial layers of the corneal epithelium were lost. The reduction in epithelial thickness after prolonged repeated irradiation of the cornea with UVB rays due to the loss of the superficial epithelial layers was also described by Cullen (1980) using slit-lamp biomicroscopy, light and electron microscopy as well as by Koliopoulos and Margaritis (1979) in their ultrastructural study. Cullen (1980) described that the biomicroscopically observed granules in the epithelium were the clinical manifestation of secondary lysosomes revealed by light and electron microscopy. According to this author, UVB rays break down primary lysosome

membranes to release hydrolytic enzymes, which in turn results in the destruction of some layers of the corneal epithelium. It is necessary to mention that after UVB irradiation of the rabbit cornea, increased activities of lysosomal hydrolases were detected histochemically in the anterior region of the corneal epithelium which correlated with increased levels of these enzymes in tears (Čejková 1998, 1999).

Wickert *et al.* (1999) determined the type of cell death (apoptosis *versus* necrosis) after exposure to UVB rays in the corneal and lens epithelium of the rat. As far as the type of cell death is concerned, UVB exposure causes morphological signs of apoptosis and TUNEL-positive cells were visible in the epithelium of the rat cornea. UVB-irradiated lens epithelial cells exhibited features typical for necrosis. Opacification of the lens appeared to follow the death of lens epithelial cells.

Čejková *et al.* (2001) studied the xanthine oxidoreductase/xanthine oxidase activities in rabbit corneas irradiated with UVB rays. The biochemical results showed that the xanthine oxidase proportion of total xanthine oxidoreductase increased with repeated irradiation with UVB rays. These results might indicate the conversion of xanthine dehydrogenase to xanthine oxidase. Very similar results were obtained by Srivastava and Kale (1999) in the liver after irradiation of albino mice with gamma rays. They found that the ratio of the activity of xanthine dehydrogenase to that of xanthine oxidase decreased with the irradiation procedure. Therefore, these authors suggested that xanthine dehydrogenase might be converted to xanthine oxidase. However, in the above mentioned experiments, the total activity of xanthine oxidoreductase (xanthine dehydrogenase + xanthine oxidase) remained constant at all radiation doses. In contrast, Čejková *et al.* (2001) found a significant decrease in xanthine oxidoreductase in the scraped corneal epithelium after irradiation procedures with UVB rays. Under similar experimental conditions UVA rays had much less damaging effect (see Figs 1 and 2). The release of xanthine oxidoreductase from the corneal epithelium extracellularly into tears and/or the denaturation of some enzyme molecules, particularly those due to the prolonged repeated irradiation of eyes with UVB rays, were assumed to be responsible. In this connection it must be pointed out that profound morphological disturbances in the corneal epithelium repeatedly irradiated with UVB rays were found by Koliopoulos and Margaritis (1979) and Cullen (1980).

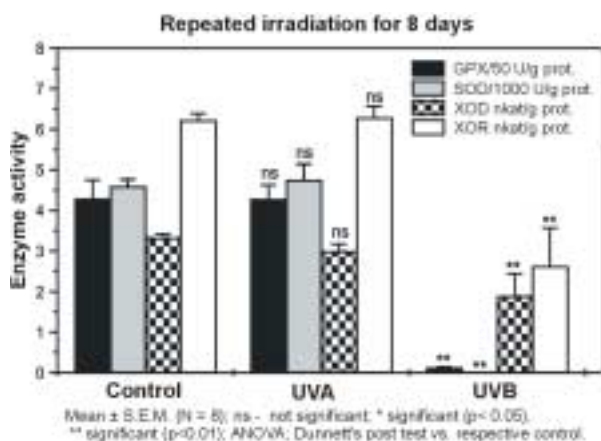


Fig. 2. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), xanthine oxidase (XOD) and xanthine oxidoreductase (XOR) in the scraped epithelium of a rabbit cornea repeatedly irradiated with UVA or UVB rays for 8 days. The open eyes were irradiated with UVA lamp or UVB lamp (Illkirch Cedex, France; 312 or 365 nm wavelength, 6 W) from a distance of 0.03 m. Only corneas were irradiated; the rest of the eye surface was protected from UV rays. The animals were irradiated for 5 min once daily, for 8 days. (For more details on experimental conditions and biochemical determination of enzyme activities, see Čejková et al. 2000 and 2001). Longer irradiation with UVB rays dramatically decreased the activities of all the enzymes investigated, mainly the activity of GPX and SOD. In contrast, the enzyme activities did not significantly change after UVA rays.

Decrease of antioxidant enzymes in the cornea after UVB irradiation

The total level of superoxide dismutase activity is much higher in the normal corneal epithelium than the activity of catalase (and catalase activity is higher than the activity of glutathione peroxidase) (Crouch *et al.* 1988). Čejková *et al.* (2000) found that in the irradiated epithelium, even after 4 days of repeated irradiation of the rabbit cornea with UVB rays, the catalase activity decreased more rapidly than the activity of superoxide dismutase. Biochemical findings concerning glutathione peroxidase also showed that the activity of this enzyme decreased in the corneal epithelium repeatedly irradiated with UVB rays for 4 days more rapidly as compared with the superoxide dismutase activity. The results suggested

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that a more profound decrease in catalase and glutathione peroxidase (hydrogen peroxide scavengers) than in the superoxide dismutase activity (an enzyme generating hydrogen peroxide as a by-product of superoxide cleavage) in the corneal epithelium increased the danger from hydrogen peroxide to the eye. The longer irradiation procedure (UVB rays) evoked a dramatic decrease in all the antioxidant enzymes studied in the flat corneal epithelium (Fig. 2). Our results are in accordance with Yis *et al.* (2002) studying the influence of corneal photoablation with argone fluoride 193 nm excimer laser on corneal glutathione peroxidase. The amount of glutathione peroxidase was significantly decreased.

Conclusions

Under physiological conditions, antioxidants are balanced with the formation of reactive oxygen species at a level at which these compounds can play their physiological roles without any toxic effects (Halliwell 1991). Furthermore, there exists a balance in the normal cornea between prooxidants and antioxidants. The cornea, together with the aqueous humor, protects the inner parts of the eye against the damaging effect of UV rays and the reactive oxygen species generated by them. This is possible due to the effective antioxidant protective mechanisms. The danger to the eye appears during increased irradiation of the eye with UV rays. The antioxidant protective mechanisms are overwhelmed, leading to a prooxidant/antioxidant imbalance. As has been shown in our experiments, this danger appears mainly after the exposure to UV rays of shorter wavelength (UVB rays) (Figs 1 and 2). The lack of corneal antioxidants is very probably an important factor enabling the development of oxidative eye injury.

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