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In vivo studies on the effect of UV-radiation on the eye lens in animals

ALFRED R. WEGENER
Department of Experimental Ophthalmology, University of Bonn, Bonn, Germany

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Abstract. Ultraviolet light is a non-ionizing radiation that induces photochemical reactions in the tissue. Its spectral A and B ranges are partially absorbed by the cornea and/or lens thus causing damage on the cellular, cell physiological and molecular level. UV-A does not seem to damage the cornea permanently and its effects in the lens have a very prolonged latency period. Typical reactions of the cornea are oedema, punctate keratitis (photoelectric keratitis) and neovascularization. In the lens all reactions that could be evidenced, were located in the epithelium and in the outer cortical fiber cells. *In vivo* UV-A induces swelling and slight vacuolation of the anterior suture system, but apart from these transient effects, only very limited permanent damage could be demonstrated. UV-B induces the formation of an anterior subcapsular cataract, starting also with vacuolation of the suture system. These morphological characteristics can be visualized at the slitlamp microscope. Histologically, sutural irregularities (UV-A) and epithelial hyperplasia with capsular multiplication (UV-B) as well as desintegration of the anterior suture system could be observed. Patho-physiologically, a reduction of lens fresh weight (UV-B) as well as changes of the equilibrium of reduced and oxidized glutathione (GSH/GSSG) could be demonstrated. On the protein-biochemical level, changes in the ratio of water-soluble versus water-insoluble protein could be evidenced, as well as effects on specific crystallin fractions, namely α-crystallin. In addition, the appearance of a newly synthetized 31 kDa protein could be demonstrated in UV-B irradiated mice.

Introduction

Ultraviolet radiation is characterized as a range of wavelengths of the electromagnetic spectrum between visible light and X-irradiation. It is not part of the ionizing radiation spectrum, as it needs to be absorbed to be effective. Classically, it is separated into 3 subranges, UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm) [1]. Subrange A and B are transmitted in increasing magnitude (UV-A $\rightarrow$ UV-B) by the global atmosphere, whereas UV-C is fully absorbed. The UV-radiation intensity per surface unit (J/cm^2) varies considerably depending on the following factors: a) quality of the ozone layer of the terrestrial atmosphere, b) geographical position and altitude above sea level, c) time of the day (position of the sun above the horizon, d) weather (clear or cloudy sky); e) degree of pollution of the local atmosphere [2–4]. Even if all these aspects have been taken into consideration, it remains very difficult to determine the actual dosage that hits the surface of the eye. Shielding effects of hats, sun glasses or open shades as well as

the surface reflectance characteristics, can influence the dosage distribution in a group of outdoor workers dramatically [5, 6]. In addition to as well as apart from the above mentioned influencing factors, tissue-specific reactions of the cornea and lens have to be taken into consideration in talking about UV-damage to the eye. If by any reason UV-C would hit the eye, it would be fully absorbed by the cornea, causing severe burning and destruction of this tissue. UV-B is partially absorbed ($\lambda < 300$ nm) c.q. transmitted ($\lambda > 300$ nm) by the cornea [6, 7], thus causing tissue damage that can be in part reversible due to the marked healing potential of the cornea. The absorption curve is influenced by the age of the individual, i.e. the transmission increases with age reaching a peak of 80% at 380 nm [8]. UV-A is almost completely transmitted by the cornea causing no acute damage to it. The lens has different absorption/transmission characteristics: nearly all wavelengths < 310 nm are fully absorbed, whereas wavelengths > 310 nm are transmitted, the transmission reaching a peak of 90% at 360 nm. The transmission characteristics of the human lens change with age too. Transmission decreases for radiation at wavelengths between 350 and 400 nm, whereas it increases towards 300 nm [1]. Apart from the age, the absorption characteristics of the cornea [9] and the lens are highly species-specific. In talking about ultraviolet radiation damaging the lens, two other factors have to be taken into consideration, the geometry of the eye ball and its position in the orbit, i.e. how well it might be shielded against radiation or exposed to it from above or below by parts of the orbit or forehead and the distance between the cornea and the anterior lens surface (= depth of the anterior chamber). The anterior chamber is filled with the aqueous humour and the penetration depth of UV in water is reciprocal to the distance from the water surface. By mentioning all these aspects of UV radiation and the eye, it is the intention of the author to highlight all factors and facts that are potentially involved in the complex interrelation between the external influencing factor UV and the target organ eye to demonstrate how difficult it is to set up experiments that are suitable to study UV damage in the eye.

Material and methods

All experiments reported and discussed in this paper, have been performed in young rodents. This comprises pigmented (Brown-Norway, BN) and albino rats (Sprague-Dawley, SD) and pigmented mice. Radiation sources for UV-A and UV-B were adapted from dermatology equipment (Waldmann PUVA 800), where they are used for PUVA therapy. UV-irradiation was performed in mydriasis every day for UV-A (1 J/cm^2/day) and every other day for UV-B (0.2 J/cm^2/2 days), using 1% Atropin as mydriatic agent. The radiation scheme

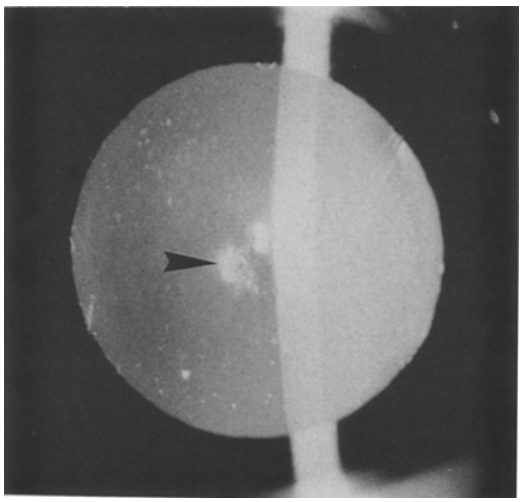

Fig. 1. Slitlamp micrograph of an anterior polar cataract (arrow) induced by UV-B irradiation over a period of 8 weeks.

was continuously applied for a period of 2 months minimum. The effects on the tissue *in situ* were monitored with a Zeiss photoslitlamp microscope and documented with a Scheimpflug camera, but the densitometric results are reported elsewhere. For histological investigations lenses were fixed and embedded according to standard methods [10] and biochemical examinations were performed in fresh lens extracts as well as extracts of distinct lens layers (Bonn Freeze Sectioning technique [11, 12]).

Experimental results

All data presented in this paper are *in vivo* data from experiments in rodents where their corneas and lenses have been irradiated *in situ* in their natural environment. Experiments with isolated lenses or lens cells in culture are reported by Hightower and McCready [13] and others.

In situ, the first effects of UV-A and UV-B are seen in the anterior suture system as faint vacuolations. Whereas UV-A in our experiments did not produce further effects, UV-B caused the formation of an anterior polar cataract right in the center of the suture lines (Fig. 1). The effects induced by UV-B-irradiation in the lenticular tissue appear on 3 levels: 1) alterations in the morphology of the lenticular epithelium and desintegration of the superficial cortical fibers in the anterior sutural complex; 2) on the physiological level; 3) on the molecular level of the DNA and on the level of the mitochondria.

Fig. 2. Central epithelial hyperplasia that is growing into the anterior suture system (arrow). The fiber cell tips are swollen and desintegrated.

The morphological alterations of the lenticular epithelium typically started as disturbances of the regular arrangement of cells in the pupillary area, accompanied by swelling of cells and desintegration of the epithelial-fiber cell interlace. In a next step, the mitotically quiescent cells in the polar region of the epithelium start to proliferate again, forming an epithelial multilayer with capsular duplications (Fig. 2). Under continued irradiation, the multilayering grows into a tumorlike epithelial structure that invades the anterior sutural system (Fig. 3). The intercellular space of the suture system becomes

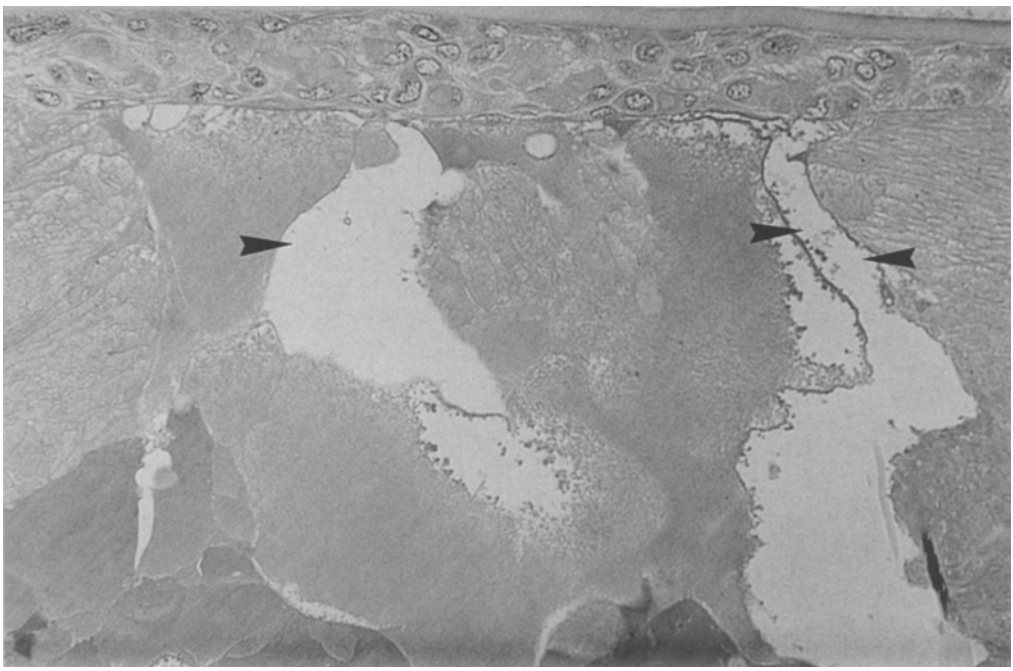

Fig. 3. Peripheral part of the polar cataract. The epithelial multilayering is clearly discernable; the sutural gap (arrow) is filled with liquefied cellular debris.

a larger gap which tends to fill up with cellular debris or intercellular liquid [14]. The tips of the fiber cells either retract or are pushed back by the liquified debris. Parallel to this, necrotic cells and cells with pycnotic nuclei are found in the epithelium. Even in mydriasis, the preequatorial parts of the epithelium remain shielded such that no alterations were found within an 8-weeks irradiation period in this region (Fig. 4). With respect to the different effects of UV-A and UV-B on the lens it is evident from the irradiation experiments that UV-A for a long interval is a sub-threshold noxious factor, whereas UV-B is a direct cataractogenic factor [15]. There is no indication, however, that their effects on the morphology of the lenticular epithelium and superficial fiber cells are different. Combined with diabetes, UV-A showed a co-cataractogenic potential, by accelerating the appearance of diabetic alterations which then mask the typical UV-radiation effects in the epithelium [16, 17]. The combination of UV-A or UV-B with X-irradiation provided no obvious interactive effects [18, 19]. In combination with certain drugs like the photosensitizer 8-Methoxypsoralen (8-MOP), both agents, the drug and UV-A, acted as syncataractogenic factors. Neither the drug nor UV-A in single

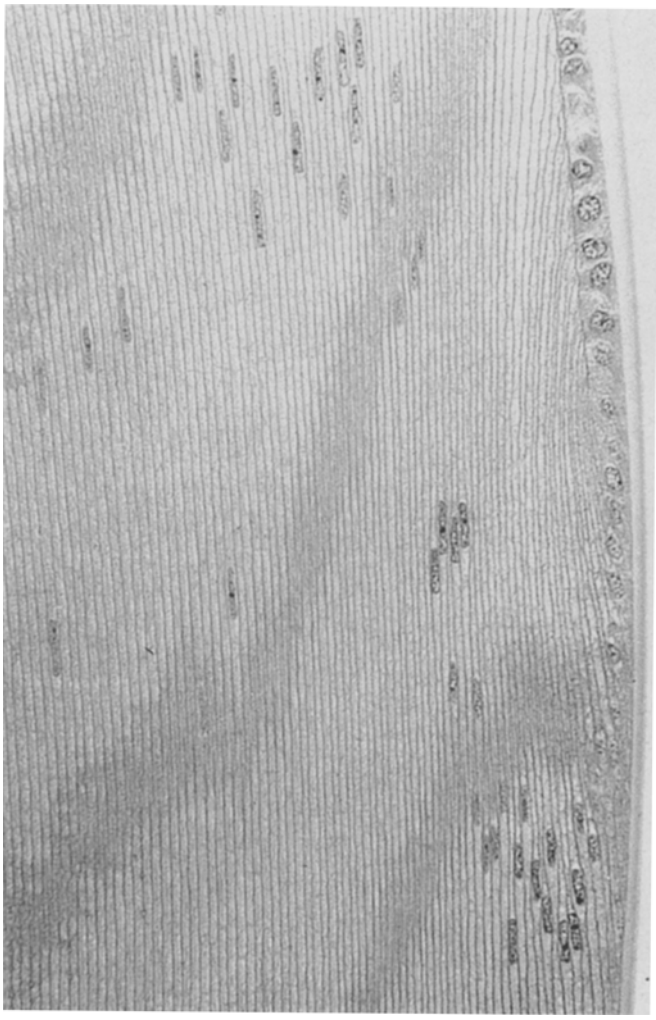

Fig. 4. Nuclear bow region of a rat lens, UV-B-irradiated for 8 weeks. With the exception of an irregular arrangement of cell nuclei, the bow region is apparently normal.

application induced changes of lens transparency; in combination, however, they triggered the formation of typical cortical cataracts [20, 21].

On the physiological level, several of the effects observed were transient and reflected a defense reaction towards the insult, a small number was permanent. The data for the lens fresh weight, however, did demonstrate an obvious difference between UV-A and UV-B. UV-A irradiation did not affect lens fresh weight [18] whereas UV-B decreased it as well in single application as in combination with X-irradiation for example (Fig. 7a) [19],

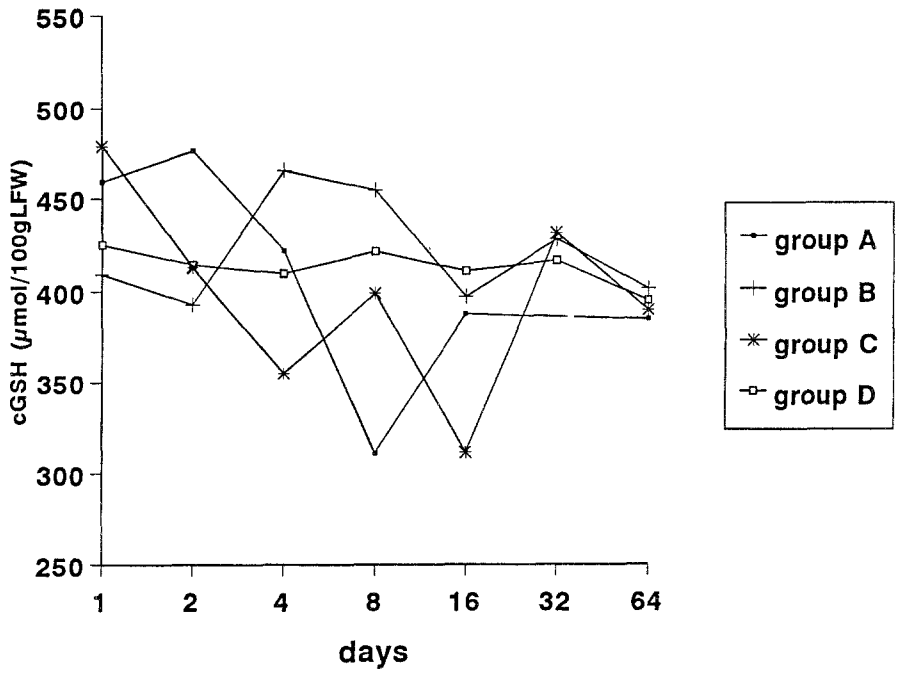

Fig. 5. Concentration of GSH (in µmol/100 g lens fresh weight, LFW) [18] in Sprague-Dawley rats monitored over an experimental period of 64 days. Group A: X-irradiation; group B: UV-B irradiation; group C: combination of X-rays and UV-B; group D: control.

although this effect could not be found consistently [22]. The concentrations of reduced (GSH) and oxidized (GSSG) glutathione in the lens showed a typical defense reaction: GSH concentrations increased in the beginning of the irradiation period, but later on oscillated around the values of the control group (Fig. 5) [23]. At the end of an 8-weeks irradiation experiment, neither the concentration of GSH nor that of GSSG was significantly different from the values in unirradiated animals (Figs. 6a, 6b). X-irradiation, however, significantly decreases GSH concentrations (Fig. 7d), whereas GSSG concentrations remained unchanged (Fig. 7e). Surprisingly, the specific activity of glutathione reductase showed a trend to decrease during the UV-radiation treatment (Fig. 7b), whereas the specific enzyme activity of GPX remained unchanged (Fig. 7c). Last but not least, a general shift of the ratio water-soluble (WS) / water-insoluble (WI) crystallin towards the increase of the insoluble fraction could be found in UV-B treated animals, but not in those treated with UV-A. α-Crystallin seemed to be primarily involved in this process [19]. In addition, in a recent experiment it could be shown, that mitochondria are a target for UV-B and that a novel 31 kDA protein band appears in the water-soluble fraction of the crystallins in UV-B irradiated

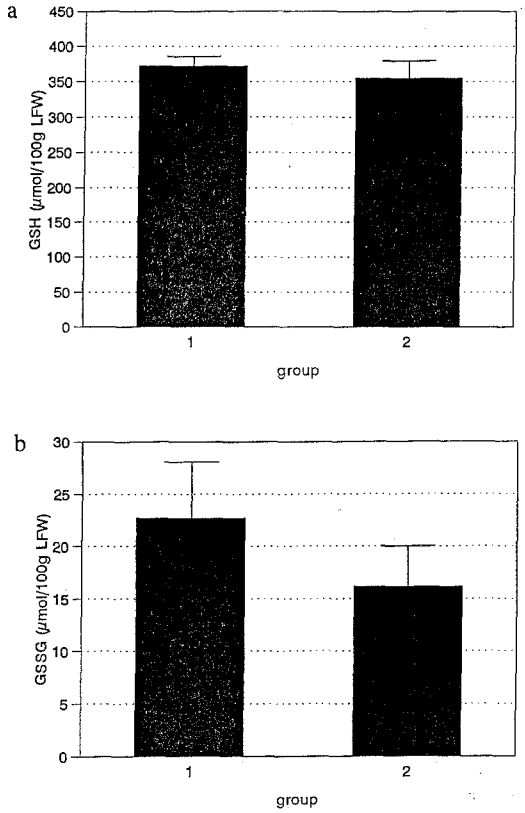

Fig. 6. Concentrations (in µmol/100 g lens fresh weight, LFW) of GSH (a) and GSSG (b) in lenses of UV-B irradiated (group 2) versus untreated BN rats (group 1) [22].

mouse lenses [24]. The nature of this protein still needs to be investigated in more detail.

Results and data from the literature

Considerable efforts have been put into the investigation of UV-damage on the molecular level. Besides photochemical reactions induced by UV in proteins and other cell constituents, aromatic aminoacids in the cellular DNA are UV-absorbing themselves [1], namely phenylalanine, tyrosine, tryptophane, cysteine and cystine. Their energy-absorbing characteristics are the reason why unscheduled DNA synthesis could be found in isolated lens epithelia after UV-irradiation by Jose and Yielding [25]. The absorbed UV energy can induce disruption of hydrogen bonds, formation of stable dimers, protein-DNA aggregates as well as single strand breaks. It is obvious that these alterations and their corresponding repair mechanisms play an important role

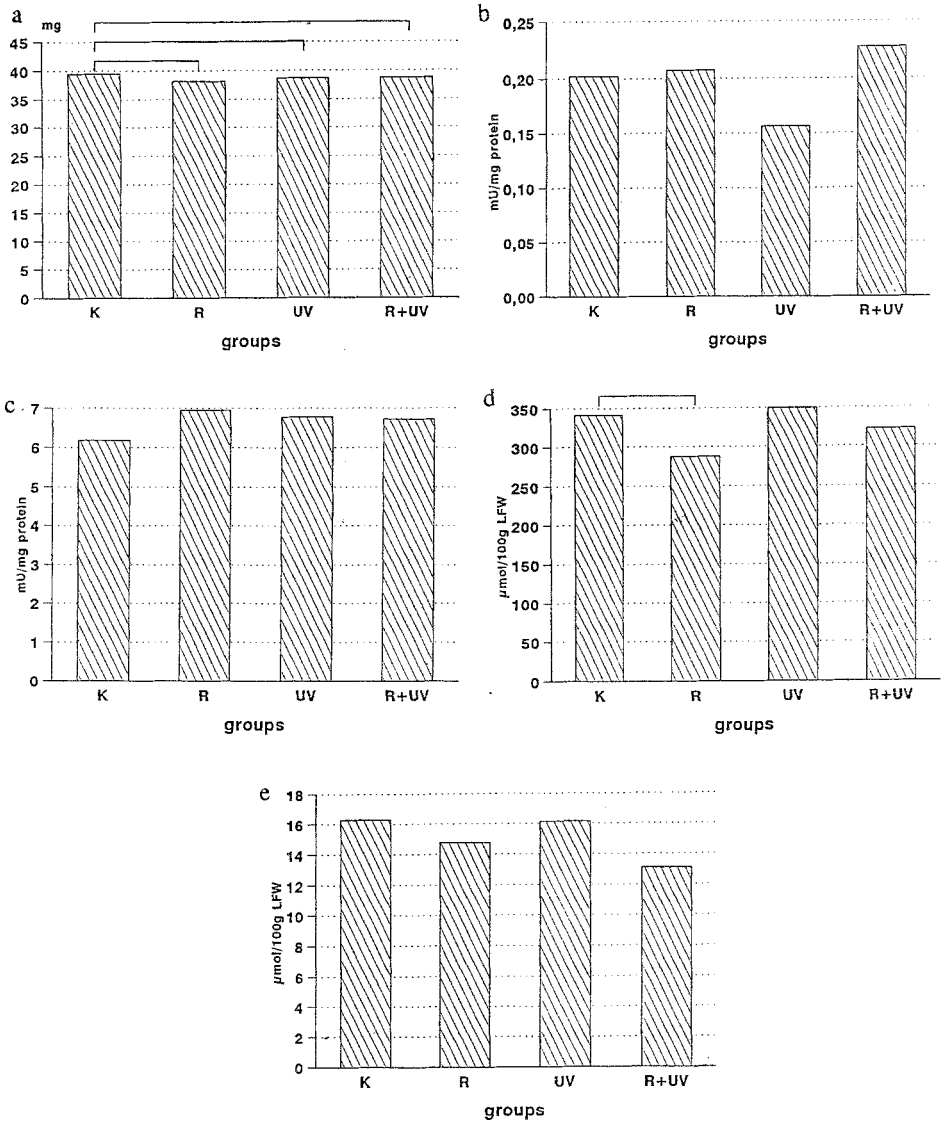

Fig. 7. Lens fresh weight, LFW (panel a); specific activity of glutathione reductase, GR (panel b) and glutathione peroxidase, GPX (panel c) and concentrations of reduced glutathione, GSH (panel d) and oxidized glutathione, GSSG (panel e) [19]. K = control; R = X-irradiation; UV = UV-B irradiation; R + UV = combination of both noxae. Bars indicate significant differences ($2p \leq 0.05$).

as short-term and even more so as long-term factors involved in radiation-induced cataractogenesis [26]. The whole spectrum of photochemical and photobiological effects on membranes, proteins and other cell compounds that can be induced experimentally by UV-A and UV-B in the lenses of

different species, predominantly rodents, is explained in detail by Dillon [27] and Zigman [28].

Conclusion and perspectives

The rodent experiments decribed above and the data summarized from the literature clearly evidence that the epithelium is the primary target tissue for UV-damage in the lens. This is already visible during slitlamp microscopical investigation, where the formation of an anterior polar cataract right underneath the capsule can be observed. On the subcellular level the primary targets obviously are the DNA and the mitochondria. On the physiological level, the balance of GSH versus GSSG apparently is the parameter of immediate reaction of the tissue to the photochemical / photo-oxidative stress caused in part by UV-A, but more prominently by UV-B. Apart from the acute effects observed morphologically in the epithelial cells and physiologically in the equilibrium GSH/GSSG, longterm effects of UV-irradiation most probably occur on the level of protein denaturation. They start from those aminoacid residues that by absorbing ultraviolet light are transformed into chromophores. Such processes can hardly be seen in rat and mouse or rabbit lenses, even in long-term experiments, but the lenses of several squirrel species seem to be a relevant model [28]. In comparing the results obtained from experimental studies to those from clinical studies in human subjects, it is evident that the acute effects investigated in detail in animal models are rarely found in human lenses. All aspects of human cataractogenesis that are believed to be related to ultraviolet radiation effects [29] are more obviously correlated to chromophore formation and protein denaturation, which most probably occur in the anterior and posterior axial cortex and nucleus. Those cases where heterogeneous cortical cataracts are attributed to UV damage, however, could be cases where an acute damage to epithelial cells or their DNA, respectively, caused maldifferentiation of fiber cells after a longer latency period, that gave rise to opaque areas in the cortex.

In addition, further experiments will be needed to investigate changes in the transmission properties of the UV-damaged cornea, as it could be shown that corneal transmission in the range of 315–415 nm decreased under the influence of ultraviolet light [30]. The importance of this finding is underlined by the observation that the sensitivity of the cornea and lens to acute UV-damage seems to decrease with age in rats [31].

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Address for correspondence: A. R. Wegener, Department of Experimental Ophthalmology, University of Bonn, Sigmund-Freud-Straße 25, D-53105 Bonn, Germany
Phone: (228) 287 5625 Fax: (228) 287 5668