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Development and Repair of Cataract Induced by Ultraviolet Radiation

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Development and Repair of Cataract Induced by Ultraviolet Radiation

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ABSTRACT

Development and repair of cataract induced by ultraviolet radiation

Background.

Several epidemiological investigations show a correlation between cataract development and the dose of ultraviolet radiation (UVR) received. It is experimentally well established that exposure of animal eyes to UVR induces cataract. Most cataracts develop as a gradual increase in lens opacity. Despite this, current estimations of toxicity for cataract are based on the concept that cataract is a binary event. Moreover, current exposure limits for UVR are based on subjective inspections with slit lamp microscopy.

Purpose.

The first purpose of the present study was to determine a statistically defined maximum acceptable dose for ultraviolet radiation-induced cataract based on quantitative data of forward light scattering in lenses. The second purpose was to find possible explanations for light scattering by investigating the morphology and the refractive index distribution in the lens. The third purpose was to describe the development of cataract after UVR on the cellular level.

Methods.

Six-week-old, female Sprague-Dawley rats received UVR unilaterally in vivo. The radiation from a high pressure mercury lamp was collimated, passed through a water filter and an interference filter or a monochromator ($\lambda_{\text{MAX}} = 300 \text{ nm}$), and projected onto the cornea. The exposure time was 15 min. The exposure dose ranged between 0.1 and 20 kJ/m² and the animals were kept between 6 hours and 32 weeks after exposure. The extracted lenses were photographed and forward light scattering was measured. Other methods included light microscopy, fluorescence microscopy, transmission and scanning electron microscopy, freeze-fracture and microradiography.

Results and Conclusions.

From a long-term experiment, it was concluded that UVR-exposed lenses scatter light more than their contralaterals and that a higher dose induces more light scattering. After exposure to 5 kJ/m², the mean forward light scattering remains unchanged between 1 and 32 weeks. Earlier observations, taken together with the current findings, indicate that the optimal time to detect low dose UVR-induced cataract is one week after exposure in rats. The intensity of forward light scattering increases exponentially with increased UVR dose between 0.1 and 14 kJ/m².

Based on this continuous dose-response, a method to determine a maximum acceptable dose to avoid UVR-induced cataract was developed. The statistically defined lower limit of pathologic light scattering is projected on the dose-response function. The dose corresponding to that point can be estimated and was suggested to be called the Maximum Acceptable Dose (MAD).

Two low dose UVR exposures with 0 or 6 h intervals between the exposures produce the same degree of lens opacification. When the second exposure follows 24 or 48 h after the termination of the first, lenticular damage increases. Repair processes between 24 and 48 h after exposure appear to be sensitive to UVR, and an additional exposure during this time may aggravate cataract development.

Lenses exposed to UVR grow more slowly than their non-exposed contralaterals. This decrease in lens growth was more pronounced with increasing dose. Low doses led to decreased water content in the lens whereas high doses led to swelling. At 6 months after low dose UVR exposure, no global change of the refractive index was found. However, local variations of the refractive index induce a subtle cortical light scattering.

In vivo low dose UVR induces programmed cell death which peaks 24 h post-exposure and involves the entire lens epithelium. Dead cells are removed from the epithelium by phagocytosis. This leads to disintegration of the lens epithelium, associated with flake-like opacities at the lens surface. After one week, the epithelium and the equatorial parts of superficial lens fibers contain extracellular spaces. The extracellular spaces together with locally disarranged fibers produce a corrugated opaque lens surface and equatorial opacities.

Within several weeks after exposure, the lens epithelium recovers, and new fibers develop normally. The lens fibers regain normal osmotic properties and fill up the extracellular spaces. Repair, however, is incomplete, and disarranged fibers remain in the cortex, producing a subtle shell-shaped opacity. In this way, subtle damage to the lens fibers induced by UVR may accumulate during a lifetime and contribute to the formation of cortical cataract.

Key words.

ultraviolet radiation, cataract, forward light scattering, lens ultrastructure, refractive index, dose-response, repeated exposure, apoptosis, in vivo, rat

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LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

- I Michael R, Söderberg PG, Chen E.
Long-term development of lens opacities after exposure to ultraviolet radiation at 300 nm.
Ophthalmic Res.1996;28:209-218.

- II Michael R, Söderberg PG, Chen E.
Dose-response function for lens forward light scattering after in vivo exposure to ultraviolet radiation.
Graefes Arch. Clin. Exp. Ophthalmol. 1998;236:625-29.

- III Söderberg PG, Michael R, Merriam JC,
Maximum acceptable dose of ultraviolet radiation: A safety limit for cataract.
Manuscript.

- IV Michael R, Löfgren S, Söderberg PG.
Lens opacities after repeated exposure to ultraviolet radiation.
Acta Ophthalmol. 1999;77:690-693.

- V Michael R, Vrensen G, van Marle J, Gan L, Söderberg PG.
Apoptosis in the rat lens after in vivo threshold dose ultraviolet irradiation.
Invest. Ophthalmol. Vis. Sci. 1998;39:2681-2687.

- VI Michael R, Vrensen G, van Marle J, Löfgren S, Söderberg PG.
Repair in the rat lens after threshold ultraviolet radiation injury.
Invest. Ophthalmol. Vis. Sci. 2000;41:204-212

- VII Michael R, Brismar H.
Lens growth and protein density in the rat lens after in vivo exposure to ultraviolet radiation.
Manuscript.

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ABBREVIATIONS

ACGIH	American C onference for G overnmental and I ndustrial H ygienists
CIE	C ommission I nternationale de l' E clairage (International Lighting Commission)
DNA	D eoxyribon u nucleic a cid
kJ/m^2	Physical unit for the exposure dose ($1 \text{ kJ/m}^2 = 0.1 \text{ J/cm}^2$)
SEM	S canning e lectron m icroscopy
TEM	T ransmission e lectron m icroscopy
tEDC	t ransformed E quivalent D iazemuls C oncentration
TUNEL	T erminal deoxynucleotidyl transferase (TdT) - deoxy u ridine triphosphate (dUTP) n ick e nd l abeling
UVR	U ltraviolet r adiation
UVR-A	Ultraviolet radiation between 315 and 400 nm
UVR-B	Ultraviolet radiation between 280 and 315 nm
W/m^2	Physical unit for the irradiance ($1 \text{ W/m}^2 = 100 \text{ }\mu\text{W/cm}^2$)
WGA	W heat g erm a gglutinin (binds to cell-surface glycoproteins)
λ	Physical symbol for wavelength

1. INTRODUCTION

1.1 The normal lens and cataract

Some of the oldest cells of the human body are those found in the lens nucleus. Despite their longevity, the central cells in the lens remain transparent over an entire lifetime. “Opacification of the aged lens results from the accumulation of insults to the lens from many different stresses incurred over the lifetime of an individual” (Jose 1978). For optimal vision, all lens fiber cells developing after birth must remain transparent until old age, because there is no cell turnover in the lens cortex and nucleus (Clark 1994).

1.1.1 Structure of the normal lens

Anton van Leeuwenhoek (1638-1723), a Dutch microscopist who was the first to observe bacteria and protozoa, was also the first to describe the fine structure of the lens (Merriam 1996). In 1674, he wrote that the bovine lens was made up of “orbicular scaly parts, lying upon one another, which had their beginning out of the center” (van Leeuwenhoek 1939). He discovered that the lens consists of lens fibers which are arranged in rows and form layers.

Figure 1. Lens of a cow's eye. Front view. Drawn by van Leeuwenhoek in a letter 1684. (Reprinted, with permission, from van Leeuwenhoek (1952). Copyright by Swets & Zeitlinger BV, Amsterdam.)

Leeuwenhoek realized that there was a regular order to the fibers. He described also the start and endpoints of the fibers and how they form opposite Y-figures on either pole of the lens (Figure 1). He did not name the sutures, but he described and drew them in a letter in 1684 (van Leeuwenhoek 1952), and estimated the number of lens fiber layers and the number of fibers lying side by side along the lens equator.

Sir David Brewster (1781-1868), a Scottish physicist noted for his experimental work in optics and polarized light, described the lens fibers as a regular transparent lamina of uniform thickness (Brewster 1816) (Figure 2). He found that the fiber is flat in cross-section and not round as described by van Leeuwenhoek. Brewster already used an optical diffraction technique to estimate the fiber dimensions and found that the fiber thickness is at least five times less than its maximum breadth. Brewster discovered the lateral binding of the lens fibers by protrusions or “teeth”.

Herman von Helmholtz (1821-1894), a German scientist and philosopher and the inventor of the ophthalmoscope, gave the first comprehensive summary on refraction and image formation in the eye (Handbook of Physiological Optics (1867)). This included also more detailed knowledge on the lens structure as for instance the hexagonal shape of the fiber cross-section.

Allvar Gullstrand (1862-1930) was appointed the first Professor of Ophthalmology at Uppsala University in 1894. He continued the work of Helmholtz and made major contributions to the theory of light refraction in the eye. This included a solution for calculating the refraction in the crystalline lens, whose refractive index varies from layer to layer. In 1911, Gullstrand received the Nobel Prize for his work on the dioptrics of the eye. Gullstrand introduced an eye model with a lens consisting of an inner nucleus with a high refractive index (1.406), which is surrounded by an area with a lower refractive index (1.385). This was a three-layer model with theoretical values for the refractive index.

The detailed distribution of the refractive index in the human lens was first measured quantitatively about 20 years ago (Fagerholm et al. 1981, Philipson & Fagerholm 1981). These studies showed a constant refractive index of about 1.41 in the lens

center occupying about 70% of the central lens diameter. The refractive index decreased almost linearly from this central core towards the lens periphery with a subcapsular refractive index of 1.37.

Figure 2.

Structure of the human lens.

(Reprinted, with permission, from van Heyningen (1975). Copyright by the artist Carol Donner, Renards Represents, New York and Scientific American, New York.)

1.1.2 Lens growth and cell division cycle

Growth of the lens begins with the division of the epithelial cells in a circular band near the equator (Figure 2). The new cells elongate to form fibers that wrap around the periphery of the lens and meet at the sutures (van Heyningen 1975). All epithelial cells are capable of mitosis but mitotic activity is greatest in the peripheral region known as the germinative zone (Sallman 1952).

While epithelial cells maintain their replicative activity, prospective fiber cells stop DNA synthesis and mitosis. Lens fiber cell nuclei undergo pycnosis, degenerate and disappear (Modak 1972) (Figure 3). Gap junctions for water and ion transport develop in this fiber maturation process and the fibers lose their organelles at the same time.

Modak (1972) uses the cell division cycle to explain lens fiber differentiation (Figure 3).

The cell division cycle is the fundamental means of cell reproduction, to make a new individual from a single cell and to replace cells in the adult body. The standard cell cycle includes a cell division or mitosis phase (M), a DNA replication or synthesis phase (S), and two gap or growth phases (G₁, G₂). The standard sequence of the cell cycle phases is: M - G₁ - S - G₂ - (Figure 3). From G₁ phase, cells can enter a resting phase (G₀) which can last days, weeks or years (Alberts 1994b). In the lens, epithelial cells in G₁ phase can enter a terminal cell cycle with final mitosis and differentiation into fiber cells (Figure 3).

A large number of cells in a tissue are often synchronized with respect to cycle phase. The typical time for one cell cycle is about 24 h, which was also found in neonatal rat lenses (Zelenka et al. 1997). However, the length of the cell cycle may be different for different developmental stages of the lens.

Figure 3.

Terminal lens fiber cell differentiation. (Reprinted, with permission, from Modak (1972). Copyright by Munksgaard International Publishers, Copenhagen.)

1.1.3 Lens transparency and cataract

Cataract is the clinical term for reduced visual function due to optical disturbances in the crystalline lens. The word itself is of Greek origin and means downrushing or waterfall, and its use in this context probably refers to the unclear view from behind a waterfall. Cataract is one of the most common diseases that reduce visual acuity in elderly people. The prevalence of cataract approximately doubles with each decade after the age of 30. In Gothenburg, the annual number of cataract operations is about 30 per 10 000 inhabitants (Carlsson & Sjostrand 1996). In Sweden, the cost of a cataract operation including the intra ocular lens is about 750 Euro.

The transparency of the crystalline lens is dependent on the regular organization of its cells and proteins. There are two main types of potential scattering centers in the lens: proteins in the fiber cytoplasm and membrane enclosed compartments. Fluctuations in the protein density induce small particle scattering whereas membrane enclosed compartments give rise to large particle scattering. The order and regularity of the lens fiber lattice also modulates total light scattering. High orderliness of the lattice results in destructive interference of the locally scattered light and hence less total light scattering.

1.2 Biological effects of ultraviolet radiation

Optical radiation between 290 and 2000 nm is an important environmental factor for many animals, including man. Humans receive more than 70 % of their information from the environment through optical stimuli. Furthermore, light has an influence on a variety of physiological and psychological responses in the human body, such as e.g. the synchronization of biological rhythms (Hyman 1990, Wurtman 1975). Another important systemic effect is the immunosuppressive potential of UVR, which lowers the resistance against tumors and infections (van der Leun 1996).

Ultraviolet radiation can induce cell damage (phototoxic response) or alter the immune system

Physically, light scattering is due to fluctuations in refractive index. Random fluctuations of the refractive index in the lens are caused by membrane degeneration, protein aggregation, density fluctuation, phase separation, and disorientation of the cytoskeletal elements (Bettelheim 1985).

Unaltered transparency requires proper protein synthesis and a maintained equilibrium of water content and of the chemical and other constituents of the lens. Agents and factors that disturb the equilibrium induce cataract. Such factors are metabolic diseases, certain drugs, different types of radiation, genetic factors, ocular inflammation and physical injury. Age also plays an important role in cataract formation, as do nutrition, genetic factors, and race. Therefore, cataract is seen as an age-related multifactorial disease (McCarty & Taylor 1996, Müller-Breitenkamp et al. 1992, Stevens & Bergmanson 1989).

(photoallergic response). It can act directly on molecules or via a sensitizer.

Because the skin and the eye are directly exposed to UVR from the environment, they are the principal targets. Solar radiation causes erythema, synthesis of melanin and vitamin D in the skin, and changes in cellular functions in the skin, and UVR influences tumor suppresser genes. Skin carcinoma and melanoma are related to UVR exposure (van der Leun 1996).

Drugs and other agents can cause photosensitization to UVR. Photosensibilization can be used beneficially in photodynamic therapy of psoriasis or herpes, but may also be a harmful pathophysiological mechanism.

1.2.1 Pathological effects on the eye

According to the fundamental law of Grothus-Draper, only radiant energy that is absorbed by the tissue can cause a biological response. Since 1845, it has been known from experiments by Brücke (1845) that when the eye is exposed to UVR near to the visible wavelengths the radiation is absorbed mainly by the lens.

In 1883, De Chardonnet investigated the transmission of light through ocular tissue from humans and various animal species (DeChardonnet 1883). He found that the human lens absorbs all radiation below 375 nm, and the human cornea, all radiation below 300 nm. Interestingly, he also found that in the bovine lens there was a significant change in lenticular transmission depending on age. [Figure 4](#) presents the current knowledge on the attenuation of UVR on passage through the human ocular media.

The shortest UVR wavelength band of biological interest is UVR-C ($\lambda = 180 - 280$ nm). The human receives UVR-C only from artificial sources. Because it is completely absorbed by the cornea ([Figure 4](#)), this radiation causes injury only of the cornea (photokeratitis) and conjunctiva.

Somewhat longer wavelengths are called UVR-B ($\lambda = 280 - 315$ nm). UVR-B is mainly absorbed by the cornea and contributes to photokeratitis. A small fraction, however, also reaches the lens. The absorption in the crystalline lens rises between 300 and 310 nm (Sloney & Wolbarsht 1980a) and the lens absorbs all the UVR-B that passes the cornea (Förster & Busse 1991). Therefore, this radiation can be especially harmful to the lens. The UVR band with the longest wavelengths and lowest energy is the UVR-A ($\lambda = 315 - 400$ nm). Like UVR-B, this radiation may contribute to cataract, albeit to a lesser extent, as less UVR-A is absorbed in the lens. UVR-A with wavelengths between 340 and 360 nm or longer, passes through the lens and may reach the retina ([Figure 4](#)). This waveband and blue light are postulated to cause age-related macular degeneration. Other ocular diseases related to UVR are pterygium (Dhir et al. 1967), a duplicate conjunctiva growth on the cornea, and pinguecula (Taylor et al. 1988), yellow hyaline deposits close to the limbus.

Figure 4.

Attenuation of ultraviolet radiation in the human eye for different wavelengths. Numbers are the percentages of incident radiation on the cornea absorbed by each tissue. (Boettner & Wolter 1962)

1.2.2 Mechanisms of lens damage

There are multiple mechanisms through which UVR damages the lens and contributes to cataract formation.

The first lenticular target for UVR-B that passes the cornea is the epithelium ($\lambda > 300$ nm), because the lens capsule does not absorb this radiation significantly (Söderberg et al. 1996). The epithelial cells are mitotic cells that divide in the germinative zone and differentiate into fiber cells (Figure 5). These fiber cells elongate and envelop the fibers that were formed earlier in life (Jose 1978). Damage to the epithelium caused by UVR-B will therefore be mediated to growing lens fibers (Hightower 1995, Jose 1986).

The most important UVR-absorbing component in the lens is tryptophan. Other amino acids (phenylalanine, tyrosine and cystine), as well as 3-hydroxykynurenine glycoside, contribute to the absorption of UVR in the lens (Sydenham 1995, Tuffs 1991).

Absorbed UVR-photons excite lens molecules, creating free radicals which increase the oxidative stress on the lens (Figure 6). This induces damage to the DNA, proteins and lipids. UVR can induce single and double strand breaks in the DNA and can damage the bases of DNA by inducing e. g. pyrimidine dimers. This affects lens cell differentiation and protein synthesis (Jose 1978). Lipid peroxidation and the inhibition of oxidative enzymes alters membrane functions and cell metabolism and finally leads to cellular edema (Kistler & Bullivant 1989, Müller-Breitenkamp et al. 1992, Spector 1995). All these changes disturb the order and placement of the lens cells and lead to an increase of light scattering in the lens (Figure 6). There are different time scales for different types of UVR induced damage; cell misdifferentiation will take more time to induce light scattering as compared to changes that induce edema.

Figure 5.
Schematic drawing of lens fiber development.
Diagram shows a sagittal section of the nuclear bow region. The anterior of the lens is left, the equator on top and posterior right.

Figure 6.

Possible pathways for cellular damage in the lens induced by UVR. (↑... increase, ↓ ... decrease)

1.3 Environmental sources of ultraviolet radiation

More time for leisure and vacations, frequently spent in southern latitudes, coupled with a longer life-expectancy, has increased the amount of natural ultraviolet radiation exposure that people in Europe and North America receive in their lifetime (Johnson et al. 1976, Moan & Dahlback 1992, Nolan & Amanatidis 1995). This exposure is enhanced by atmospheric changes which allow a greater amount of shorter wavelength radiation to reach the earth's surface (Dobson 1963, Munakata 1995, Zerefos et al. 1995). Artificial sources of UVR have found increasing use in sun tanning and industry, e.g., sterilization, photochemistry. All of these factors have increased our total environmental exposure to UVR.

1.3.1 Terrestrial exposure

Solar radiation approaching the earth's atmosphere must penetrate the atmosphere along a slanted path. The length of the path is determined by the solar elevation angle, which varies with time of day, season and latitude (Figure 7), and is often expressed as relative thickness of the atmosphere or air mass. The air mass is the ratio of the length of the slant path to the length of the vertical path (Gates 1966). The length of this path determines the attenuation of the solar radiation that occurs by atmospheric absorption and scattering mainly due to water and oxygen. Another factor influencing the path length of the solar rays is altitude.

Figure 7.

Factors influencing global and direct solar radiation on the earth's surface.

Figure 8.

The geographical distribution of monthly-integrated UVR from 290 to 400 nm weighted by the CIE erythral action spectra.

(Adapted from Herman et al, J. Geophys. Res., Vol. 104 - D10, pp 12059-12076, 1999. Copyright by the American Geophysical Union.)

Figure 8 illustrates the distribution of solar UVR at the earth's surface. It gives the solar radiation weighted by the CIE erythral action spectrum (Figure 12) and integrated for 30 days. The data are estimated using the ozone amount, cloud transmittance, aerosol amounts, and surface reflectivity from the solar UVR backscattered from the earth's atmosphere as measured by TOMS (Total Ozone Mapping Spectrometer), and independently measured values of the extraterrestrial solar radiance (Herman 1999).

July is the month with the maximum solar radiation for the Northern hemisphere (Figure 8a) and January for the Southern hemisphere (Figure 8b). The impact of latitude, altitude and vegetation on solar UVR is visible in Figure 8. There is always a North-South difference in the UVR dose. The region of Tibet and the Himalayas (Figure 8a) as well as the Andes in South America (Figure 8b) have high values of UVR due to high elevation. The Amazonas rain forest causes low values of UVR as compared with neighboring areas at the same latitude (Figure 8).

Figure 9 shows two sample spectra of the solar UVR spectrum at different latitudes. Both spectra were measured at the annual maximum solar elevation angle for each location (Wester 1987, 1996). The entire UVR spectrum is attenuated more in Stockholm than on the Canary Islands, and the most significant difference is found between 290 and 310 nm.

Figure 9.

Measured spectral solar irradiance on the earth's surface for different latitudes. Measurements taken under a clear sky at solar noon on La Palma, Canary Islands, latitude 28°N, altitude 2350 m, solar elevation angle 85° (air mass 1.0), 30.06.1986 and in Stockholm, Sweden, latitude 59.3°N, altitude 0 m, solar elevation angle 50° (air mass 1.3), 07.07.1989 by Wester (1996).

($1\text{ W/m}^2 = 100\mu\text{W/cm}^2$)

	Total terrestrial solar irradiance (W/m^2)		Safe ocular exposure time to avoid cataract * (min)
	UVR-B (280 - 315 nm)	UVR-A (315 - 400 nm)	
Canary Islands	3.2	65.8	23
Stockholm	0.9	34.8	65

Table 1. Measured total solar irradiance on the earth's surface for different latitudes. For environmental conditions see figure 9. The safe ocular exposure time is calculated according to Pitts (1993).

(*... for constant gazing against the sun.)

Figure 10.

Theoretical spectral solar irradiance on the earth's surface for different stratospheric ozone concentrations. Calculated from relative data by Dobson (1963) for clear sky, sea level and solar elevation angle 90° using data from empirical equations by Diffey (1977) based upon the measurements of Bener (1972).

($1\text{ W/m}^2 = 100\mu\text{W/cm}^2$;

"1 mm" ozone = 100 Dobson units)

	Total terrestrial solar irradiance (W/m^2)		Safe ocular exposure time to avoid cataract * (min)
	UVR-B (280 - 315 nm)	UVR-A (315 - 400 nm)	
without ozone	12.4	55.2	6
"2 mm" ozone	2.6	54.6	30
"4 mm" ozone	1.7	54.2	48

Table 2. Theoretical total solar irradiance on the earth's surface for different ozone concentrations. For environmental conditions see figure 10. The safe ocular exposure time is calculated according to Pitts (1993). - (*... for constant gazing against the sun.)

Due to the absorption of short-wave solar radiation ($\lambda = 140 - 200$ nm) by oxygen, ozone is formed in the stratosphere. The ozone in turn absorbs solar radiation with longer wavelengths ($\lambda = 200 - 300$ nm) and is split into oxygen (Phillips 1983). In this way, the absorption of UVR by oxygen and ozone protects the earth's surface from all solar radiation shorter than 290 - 300 nm. This stratospheric equilibrium may be upset by the catalytic disassembly of nitric oxides (N_2O ; NO) in the presence of volcanic or anthropogenic chlorine, bromine or methane (Figure 7). Figure 10 shows the protective effect of the ozone layer. The ozone molecules are spread throughout a layer which is several kilometers thick. The concentration of ozone, however, is commonly described as the thickness this layer would have were it compressed against the earth's surface. The monthly average in Sweden usually varies between 2.5 and 4.5 mm ozone during the year (Wester 1993). Different stratospheric ozone concentrations affect the solar spectrum only in the UVR-B region (Figure 10 and Table 2) (Dobson 1963). The effect is, again, most prominent around 300 nm.

To illustrate the damaging potential of UVR, the time for safe constant viewing to the sun is calculated according to Pitts (1993). Solar radiation on the Canary Islands is three times more likely to induce cataract than in Stockholm. Whereas a 50% reduction of the ozone concentration would increase the risk two-fold (Table 1 and Table 2).

As the sun rays continue traveling through the atmosphere, they enter the troposphere where they are scattered and attenuated by clouds and air pollution. Although this attenuates direct solar

radiation, it may increase the global UVR due to wide angle scattering which is determined by the degree of cloudiness. Landscape and vegetation have the final influence on how much radiation is received by the earth's surface (Figure 7).

1.3.2 Social aspects

The lifestyle in developed countries has changed rapidly during the last 50 years. Weeks of holiday time and almost unlimited mobility have become the norm. And because sun-bathing and holidays in warmer climates are very popular, people are traveling more often to tropical latitudes. During a holiday to the Canary Islands a Stockholm resident gets three times as much exposure to UVR-B as at home (Table 1).

Although urbanism and a primarily indoor work environment decreased the general exposure to solar radiation overall, it may lead to a lower tolerance to UVR. Improved living standards, and increased use of drugs, and artificial components in our environment have led to an increase in allergies and may also increase the susceptibility to photosensitizers.

Increased use of artificial sources of UVR also contributes to the environmental UVR dose. The quest for beauty (tanning booths), medical and industrial applications (chemical industry, disinfection), and black lights (insect traps, discotheques) are common today. Last but not least, increased life-expectancy increases the total amount of UVR one is exposed to over one's lifespan.

1.4 Ocular sunlight exposure and corneal transmission

1.4.1 Ocular sunlight exposure

The level of ambient solar UVR is most commonly expressed as the irradiance on the horizontal earth surface. Only a fraction of this irradiance, however, reaches the anterior human eye. Our facial geometry, especially the eyebrows and eyelids protect our eyes from the direct solar rays. We also avoid exposure

with a downward normal line of sight of 15° (Slinney 1995). The reflective properties of the ground must also be considered. For example, grass reflects only 1 to 5% UVR whereas fresh snow may reflect up to 88% (Slinney 1986).

The relationship between the irradiance on the earth surface and ocular exposure is described by the ocular-to-ambient exposure ratio (Rosenthal et al. 1988b). Rosenthal et al. found this ratio to be between 2 and 17%, depending on clothes, working conditions, and time of the year.

Use, size, and shape of sunglasses, as well as their distance from the eye, are other factors that make ocular exposure difficult to estimate (Rosenthal et al. 1988a). Lateral ocular exposure may cause lens damage, as well as temporal rays which may be focused into the lens by the cornea. This latter occurrence is known as the Coroneo effect (Coroneo et al. 1991) against which common sunglasses do not protect.

Several approaches have been tried in an attempt to measure ocular exposure more directly, for instance by using dolls or by placing UVR sensitive film at different positions on the heads of subjects. An interesting approach to ocular dosimetry is the use of contact lenses with Columbia Resin 39 or polysulphone as applied by Sydenham (1995). Employment of such contact lenses will supply dosimetry data directly from the corneal plane, allowing calculation of distinct values for the ocular-to-ambient exposure ratio under different conditions.

Merriam used computer ray tracing and mechanical models of the human eye to study the refraction of light by the cornea and lens (Merriam 1996). He found that the concentration of light within the lens increases with angle of incidence and that the inferior and inferonasal lens receives more solar radiation than other sections of the lens.

1.4.2 Corneal transmission

To understand the in vivo injurious effect of UVR on the lens, knowledge on the transmission properties of the cornea is important. Dillon et al. (1999) measured the transmittance of the cornea in the mouse, rat, rabbit and human with a spectrometer, using fiber optics (Figure 11). At 300 nm, the transmittance of the mouse and rat cornea, and of the rabbit and human cornea are very similar. This is probably due to the comparable thickness of the cornea of both pairs of species (Table 3).

The data from Figure 11 were also utilized to estimate the amount of UVR used in this study that would reach the lens of the rat and human (Figure 23).

Figure 11.

Typical transmission spectra for a mouse, rat, rabbit and human cornea.

(Reprinted, with permission, from Dillon et al. (1999). Copyright by Academic Press, London.)

	Corneal thickness (μm)	Corneal transmittance at 300 nm (%)	Corneal transmittance at 380 nm (%)	Anterior chamber depth (mm)	Eye globe anterior - posterior diameter (mm)
Mouse	90..100 ^{1,2}	37 ²	81 ²	0.45 ¹	3.4 ¹
Rat	120..160 ^{2,3,4,5}	32 ²	76 ²	0.70 ⁵	6.3 ⁵
Rabbit	380..400 ^{2,6}	13 ²	71 ²	3.50 ⁷	16.0 ⁷
Human	530..550 ^{2,6}	10 ²	63 ²	3.50 ⁷	24.0 ⁷

Table 3.

Dimensions of the anterior eye of different mammals and corneal transmittance at two wavelengths in the UVR.

1 (Remtulla & Hallett 1985)

4 (Behar-Cohen et al. 1998)

7 (Davson & Graham 1974)

2 (Dillon et al. 1999)

5 (Chaudhuri et al. 1983)

3 (Sandvig et al. 1997)

6 (Li et al. 1997)

1.5 Earlier research on ultraviolet radiation and cataract

The transmission properties of ocular tissue were first scientifically investigated in the middle of the 19th century (Brücke 1845, DeChardonnet 1883). In Stockholm, at the beginning of the 20th century, Widmark compared the ocular injury occurring in people who have been struck by lightning with reports of snowblindness (photokeratitis) as well as with experiments by Hess (1868) who placed electrical discharges close to the eyes of rabbits. Widmark noticed similarities in the ocular damage and, knowing that UVR is produced by an electrical discharge, he postulated that UVR caused photokeratitis. From these findings, he speculated that ultraviolet radiation damages the cornea and lens (Widmark 1889).

Widmark tested this theory by exposing a pig lens placed on his arm to UVR from a carbon arc lamp. After exposure, his skin showed severe erythema except in the place that had been covered by the pig lens. He then postulated that the radiant energy of the UVR absorbed by the pig lens damages the lens (Widmark 1891). In later experiments in rabbits, he found lens epithelial damage and swollen lens fibers, but no damage of the lens equator, following controlled exposure to UVR (Widmark 1901).

Verhoeff et al. gave a review of the knowledge concerning “the pathological effects of radiant energy upon the eye” at the beginning of the 20th century (Verhoeff et al. 1915/16). Since that time, there have been many experimental investigations and epidemiological studies of a possible relationship between UVR and cataract.

Epidemiological studies have the advantage of investigating the influence of UVR exposure over the long term, with results which can be directly applied to the human in a general sense. It is still difficult, however, to calculate the dose of UVR exposure for each individual subject over decades of exposure. Although dosimetry in experimental exposure on animals can be well controlled and co-factors for cataract can be excluded, the results must then be translated to the human.

1.5.1 Epidemiology of cataract

Epidemiological cataract research has always been challenged by the variety of factors influencing cataract development and by the difficulty of quantitative measurement of the optical properties in vivo.

Zigman et al. (1979) compared the prevalence of cataract in Manila, Florida, and New York and grouped the individuals according to primarily outdoor or indoor activity. He found no dependence of cortical cataract on latitude ("more sunlight"), however there was such a dependence for brunescant cataract. Later, Brilliant et al. (1983) found a negative correlation between altitude, and a positive correlation between sunlight hours, and cortical cataract.

Taylor (1990) looked at 800 waterman in the Chesapeake Bay study. He estimated the individual UVR dose for each subject and found a correlation between individual UVR-B dose and cortical cataract. Cruickshanks et al. (1992) found only for male subjects a similar correlation in the Beaver Dam study. Bochow (1989) also linked posterior subcapsular cataract to UVR-B in a case-control study in Maryland. West et al. (1998) found an association between ocular exposure and increasing odds of cortical opacity, despite the relatively low ocular exposure to UVR-B in the population investigated. This carefully designed study, the Salisbury Eye Evaluation project, included 2520 community-dwelling elderly people.

Follow-up investigations of the Beaver Dam study (Klein et al. 1992) and of the Chesapeake Bay study (Schein et al. 1994) found an association of cortical cataract with the inferonasal quadrant of the lens. Also other studies report that cortical opacities were found more often inferonasal than elsewhere in the lens (Adamsons et al. 1991, Sasaki 1997). It would be difficult to explain this finding on the basis of nutritional, metabolic or aging effects. But the finding favors the theory that this type of cataract is caused by solar radiation.

There are a few reported cases in which people who received significant UVR doses from artificial sources subsequently developed cataract which could be correlated to this exposure. Lerman reported three patients who received 0.3 - 0.4 W/m² UVR ($\lambda = 300 - 400$ nm) for about 200 h over an 18 month period related to their

occupation. He found posterior subcapsular and zonular cortical opacities in one or both eyes of the patients (Lerman 1980).

Müller-Breitenkamp reported anterior and posterior subcapsular opacities in both eyes of a 65-year-old patient 30 years after unintended exposure to UVR. The patient received 0.7 - 2 kJ/m²/day UVR-B and C during the winter season over 15 years. The time that had elapsed since exposure was confirmed by measuring the distance between the lens capsule and the cortical opacities (Müller-Breitenkamp et al. 1997).

1.5.2 In vivo experiments

UVR-B is known to have a higher potential to damage the lens than UVR-A (Jose & Pitts 1985, Wegener 1994). Exposure to 30 kJ/m² UVR-B in vivo induces an exponentially declining increase of sodium in the lens ($t_{0.5} = 38$ h) and a concomitant decrease of potassium which leads to a transient net increase of solute concentration peaking around 24 h after exposure (Söderberg et al. 1989). The increase of solute leads to an uptake of water that also peaks 24 h after exposure (Söderberg 1989). In parallel, the intensity of forward light scattering increases exponentially declining ($t_{0.5} = 19$ h) (Söderberg 1990b). Morphologically, these events correspond to swelling and disruption of first lens epithelial cells and later cortical lens fibers (Breadsell et al. 1994, Schmidt et al. 1988, Schmitt 1988, Söderberg 1988, Wegener 1994).

Investigations of the UVR action spectra in the rabbit by Pitts et al. (1977) showed that the wavelength 300 nm is most cataractogenic. The same group identified the UVR dose of 5 kJ/m² at 300 nm as the threshold for permanent lenticular damage. The dose-response for cataract after in vivo UVR-B was investigated by Söderberg and Löfgren (1994). They found the damage after UVR to be proportional to the logarithm of the dose.

Chronic exposure to UVR-A can induce epithelial damage and abnormalities in the anterior pole (Zigman et al. 1991).

1.5.3 In vitro experiments

In vitro exposure of bovine, rabbit, and human lenses to UVR also lead to cataract formation and caused epithelial cell damage and decreased enzyme activity (Andley & Weber 1995, Andley et al. 1990, 1994, Stuart & Doughty 1995). The effects of UVR-A and B on cultured animal lenses were compared by Hightower et al. (1993) and by Stuart et al. (1994). Both found a much greater response to UVR-B than UVR-A. Cultured bovine lenses showed a significant decrease in transmittance one week after a dose of 0.6 kJ/m² UVR-B, but not after a dose of 0.3 kJ/m². In cultured rabbit lenses, 30 kJ/m² UVR-B induced opacification (the lenses “became opaque”), whereas 3 kJ/m² had “little effect” one day after exposure. Significantly higher doses of UVR-A had no detectable effect on transmittance (Stuart: 14.4 kJ/m² after 20 days and Hightower: 240 kJ/m² after 4 days).

1.5.4 Current estimation of risk for cataract

Most cataracts develop as a gradual increase of lens opacity. Despite this, the estimations of risk for cataract today are founded upon the idea that cataract develops as a binary event. Furthermore, current exposure limits for UVR are based on subjective observations with slit lamp microscopy.

Most often, acute toxic effects are expressed as percent incidence occurring in an exposed population (Brown 1980). The dose causing a certain incidence of effect is called ‘Effective Dose’ (ED). The percentage of the population showing the effect is given as subscript. For example, if an effect is produced in 10% of the exposed population, it is written as ED₁₀.

The most common method for estimating the Effective Dose is the so-called Probit Analysis, developed by Finney (1971). This method, based on a quantal response, describes ‘effect’ or ‘no effect’ after a certain dose of a toxic agent.

The response is illustrated by plotting the proportion of the sample responding (ordinate) as a function of dose (abscissa). Such dose-response curves have a sigmoid shape. In Probit Analysis, the ordinate is substituted by a Probability Unit (‘Probit’) and the abscissa is converted to log-scale (Brown 1980, Finney 1971). By doing so the sigmoid curve is transformed into a straight line, and the Effect Dose for different percent of incidence can be easily estimated.

1.5.5 Exposure limits for UVR

One way to compare the biological effectiveness of UVR sources which have different spectra is the use of the erythral action spectrum adopted by the Commission Internationale de l’Eclairage (CIE Research Note 1987) ([Figure 12](#)).

The American Conference for Governmental and Industrial Hygienists (ACGIH, USA) has adopted a relative spectral effectiveness curve for UVR ([Figure 12](#)) which is also recognized by the International Radiation Protection Association (IRPA). Ocular action spectra data upon which this effectiveness curve is based originate from the experiments of Pitts et al. (1977). They exposed the eyes of rabbits to UVR of increasing doses and at various wavelengths and then observed the cornea and lens with a slit lamp microscope. Using a qualitative grading system, the highest dose with no visible or only a limited number of damage signs was estimated as threshold for damage to the cornea and lens (Pitts et al. 1977).

Based on these experiments, Pitts published relative spectral effectiveness data for the cornea and the lens (Pitts 1993). His findings concerning the rabbit are given in [Figure 12](#). Knowing the absolute effectiveness at the peak of the relative curve and the absolute spectral data for a given radiation source, it is possible to calculate the duration of safe exposure to that source (Pitts 1993). Results of such calculations for solar radiation are given in [Table 1 and 2](#).

Figure 12.

Relative effectiveness of UVR for the human as adopted by ACGIH (1996), relative effectiveness to induce erythema as adopted by CIE (CIE Research Note 1987) and relative effectiveness of UVR for cataract and photokeratitis for the rabbit by Pitts (1993). The minimum dose to induce erythema is about 250 J/m^2 below 300 nm (depending on skin type and adaptation). The radiant exposure threshold for photokeratitis and for the ACGIH spectrum is 30 J/m^2 at 270 nm and for cataract it is 1.5 kJ/m^2 at 300 nm . ($1 \text{ kJ/m}^2 = 100 \text{ mJ/cm}^2$)

It should be noted that the threshold for photokeratitis is lower than that for cataract (legend of Figure 12). However, cataract is a late response with cumulative tendency while corneal UVR damage is an immediate response. “Thus repetitive ultraviolet exposures, though subthreshold for the cornea may well lead to cataract in the long run” (Bachem 1956).

2. AIMS OF THE STUDY

The aims of this study were to:

- ☐ find the optimal time point for detection of UVR-induced cataract
- ☐ determine the dose-response function for UVR-induced cataract
- ☐ develop a method for experimental estimation of the risk that UVR will induce cataract
- ☐ investigate the effect of fractionation of UVR dose on cataract development
- ☐ study apoptosis in the lens after UVR exposure
- ☐ investigate the development and recovery of lens damage after UVR exposure
- ☐ estimate the refractive index distribution in the lens several weeks after UVR exposure

3. METHODS

3.1 Experimental animals

Young adult female Sprague-Dawley rats (6 weeks old, mean body weight 165 g) were selected for all experiments. This age group was chosen to conform with an earlier short-term follow-up-study (Söderberg 1990b). At the most extended post-exposure time (32 weeks), the animals were 38 weeks old, which is about one third of the rat's natural lifespan under laboratory conditions (Waynforth & Flecknell 1992).

The rat eye is in many respects different from that of the human. Most important for the present studies are the dimensions of cornea and lens (Figure 13).

Figure 13.
Cross-section of the anterior eye of the human and the rat, drawn from histological sections.

The human cornea is about five times thicker than the rat cornea and therefore transmits less UVR (Figure 11 and Table 3). The rat lens is not as flat as the human lens (Figure 13) and the rat lens does not change its shape by accommodation. However, the general structure, growth pattern and the proteins are comparable in the rat and the human lens.

Unpigmented rats are used in the present studies. The pigmentation of the animal's lens cortex and nucleus should be of minor importance because the UVR-B as applied in the experiments is already absorbed in the very first layers of the lens (Hightower et al. 1994, Söderberg et al. 1998). In contrast, UVR-A is penetrating much deeper into the lens and some parts of this radiation are transmitted by the lens (Boettner & Wolter 1962, Lerman 1984). Therefore the pigmentation is more critical in studies of UVA-radiation (Zigman et al. 1991).

Ten minutes preceding each UVR exposure, the animal was anesthetized by intraperitoneal injection of a mixture of 94 mg/kg Ketamine and 14 mg/kg Xylazine. Five minutes after injection, Tropicamide was instilled in both eyes. After another 5 minutes, the eye was exposed to UVR for 15 minutes. The animal was exposed with a narrow beam which covered only the cornea and the eyelids of the exposed eye.

The animals were kept and treated according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

3.2 Radiation exposure equipment

The exposure system used in the experiments of Appendix I, II, V, VI and VII was based on the principle of Köhler's illumination [Figure 14a](#) and was developed by Söderberg (1990a). A high pressure mercury lamp (HBO 200 W, Osram, Germany) was the radiation source. A spherical reflector was placed behind the lamp to collect and concentrate the radiation from the backward

direction. In the forward direction, the radiation passed through a water filter which absorbed infrared radiation. The radiation then passed through a condenser system including a 300-nm-interference filter with a half band width of 10 nm. Behind this, the field aperture for the final beam was placed. The intensity of the final beam was controlled by a diaphragm in front of the projection lens.

Figure 14a.

Principle of the interference-filter-based exposure source (Appendix I, II, V, VI, VII).

(Reprinted, with permission, from Söderberg (1990a). Copyright by Munksgaard International Publishers, Copenhagen.)

Figure 14b.

Principle of the monochromator based exposure source (Appendix III and IV).

For the experiments described in Appendix III and IV, a new exposure system was designed (Figure 14b). Here, the radiation from a high pressure mercury lamp (350 W, Oriel 6286) was collimated and passed through a water filter and a double monochromator (Oriel 2x 77 250) before being projected to the cornea of the exposed eye. The monochromator was set to a wavelength of 300 nm and the entrance and output slit were adjusted to achieve a full width at half maximum of 9 nm.

The spectral distribution of both exposure systems was measured with a fiber optic spectrometer (Ocean Optics PC 2000) and is given in absolute values of spectral dose and irradiance for a total dose of 5 kJ/m² (Figure 15).

The spectra were not symmetrical, as would be expected with radiation passing through an interference filter or monochromator. The reason for that is the line shape spectrum of the mercury discharge. Because of an intense mercury line at 302 nm, the maximum intensity was found at this wavelength. Further lines from the mercury discharge at 297 nm and a strong line at 313 nm contributed to the spectrum. It should be noted that the radiation spectrum behind the monochromator is more narrow than behind the interference filter (Figure 15).

During the experiment, the irradiance at the corneal plane was measured with a thermopile (7104, Oriel, USA) with a sensitivity of 45.5 $\mu\text{V}/(\text{W}/\text{m}^2)$. The instrument was calibrated regularly by the Swedish National Bureau of Standards.

Figure 15.

Corneal spectral dose and irradiance for the interference-filter- and mono-chromator-based exposure source.

Both curves are absolute data for a total dose of 5 kJ/m² given during 15 min. The resolution of the spectra is 1 nm.

3.3 Light scattering measurements

The intensity of forward light scattering is measured with the Light Dissemination Meter (Figure 16) developed by Söderberg (1990). This instrument uses the principle of dark-field illumination, in which the illuminating light transilluminates a transparent object at such a flat angle that it cannot enter the objective aperture. If the object contains scattering particles, the transillumination light is scattered (Figure 17a).

Figure 16. Photographic view of the Light Dissemination Meter with microscope basement (dark-field illumination), rebuilt photographic camera and Ampere meter on the left.

Figure 17a. Schematic drawing of the Light Dissemination Meter. Examined lens under dark-field illumination, I = ideally clear lens, II = opacified lens, DVM = digital volt meter. (Reprinted, with permission, from Söderberg et al. (1990). Copyright by Munksgaard International Publishers, Copenhagen.)

Figure 17b. Ray tracing of the Light Dissemination Meter with dimensions and optical properties.

Light which is scattered in the forward direction at an angle of 45° reaches the objective (Figure 17b). Light within an angle of 5° is collected by the objective and is measured by a photodiode.

The scattering standard was a lipid emulsion of Diazepam (Diazemuls, KabiVitrum, Sweden), with the unit of measure, therefore, expressed as transformed Equivalent Diazemuls Concentration (μ EDC) (Söderberg et al. 1990).

A typical value for a normal rat lens is about 0.1μ EDC and for a very opaque lens about 1μ EDC. The calibration curve in Figure 18 shows that the measured current from the photodiode increases linearly with the concentration of light scatterer (Diazemuls concentration) in the area of interest between 0 and 0.8μ EDC.

The minimum detectable difference of forward light scattering as measured with the Light Dissemination Meter was 0.17μ EDC for a sample size $n = 20$ and 0.20μ EDC for $n = 15$ considering a 90% chance to detect a size difference between means with a significance level of 0.05 (Zar 1999b).

Figure 18.
Calibration curve for the
Light Dissemination Meter.

3.4 Microradiography and confocal microscopy

3.4.1 Laser scanning confocal microscopy

The Laser scanning confocal microscope was employed for three different purposes in the current study. It was used in reflective confocal mode to image fluorescence from immuno-histochemical staining of TUNEL (Appendix V) and WGA (Appendix VI). This is by far the most common application of such microscopes, collecting the fluorescent signal only from a finite confocal layer.

Laser scanning confocal microscopes are less frequently used in transmitted non-confocal mode. Here, the feature of the scanning laser was utilized to measure the transmittance of a microradiogram point by point (Appendix VII). However, because the laser ray does not travel only through the central part of the microscope optics (as in a densitometer), vignetting occurs. Vignetting means intensity loss at the edge of images. In the current study it was minimized by careful adjustment of the scanning microscope. Measurements in background images

revealed that the center-to-edge vignetting was always less than 3%, making a planned image processing to compensate for vignetting unnecessary.

The Laser scanning confocal microscope can also be utilized to estimate the thickness of a specimen section (Brismar et al. 1996). Then, the microscope is operated in reflective confocal mode and the auto-fluorescence of the specimen section is imaged as a vertical profile image (Appendix VII, figure 4). In the current manuscript version of Appendix VII, the thickness estimations were not performed perpendicularly to the section. Therefore, the thickness was overestimated which results in a systematic underestimation of the protein density. However, because this is a systematic error, it should not influence the conclusions of Appendix VII, which are drawn from the comparison of the exposed and non-exposed lenses.

3.4.2 Microradiography

It has been shown that there is a linear relationship between the refractive index and the concentration of unconjugated proteins in biological specimens (Barer & Joseph 1954). Based on this, Lindström (1955) and Philipson (1969c) continued the development of contact microradiography for the estimation of the protein density in the crystalline lens.

In microradiography, the dry mass content of freeze-dried tissue sections is estimated. The tissue

sections are placed on a metal disk together with a reference system (Figure 19). The X-ray film plate is placed behind the metal disk and is then exposed to continuous X-ray (Roentgen) radiation. The development of the film plate gives the microradiogram (Figure 19). After measuring the transmission of the microradiogram, the dry mass density in the specimen can be estimated by comparing it with the known mass of the standard film in the reference system (Lindström 1955, Philipson 1969c).

Figure 19.

Schematic representation of the quantitative microradiographic procedure to determine the total dry weight of small biologic objects. (Reprinted, with permission, from Lindström (1955). Copyright by Munksgaard International Publishers, Copenhagen.)

4. RESULTS AND DISCUSSION

4.1 Development of forward light scattering (I, II)

The lens opacities have been quantitatively described based on the intensity of forward light scattering in the lens. The development of the forward light scattering between 1 and 32 weeks is described in Appendix I. The dose-response relationship of the forward light scattering is described in Appendix II.

4.1.1 Time point for maximum light scattering

It was found that the mean difference in intensity of forward light scattering between exposed lenses and their non-exposed contralaterals;

- does not change between 1 and 32 weeks after 5 kJ/m² UVR (Appendix I)
- reaches a maximum at one week after 20 kJ/m² UVR (Appendix I)
- has an exponentially declining increase up to one week after 30 kJ/m² UVR (Söderberg 1990b).

These observations indicate that further studies of lens damage should be conducted evaluating changes which occur one week after exposure to UVR. It was not previously known that lenses exposed to 20 kJ/m² UVR are totally destroyed four weeks after exposure (Figure 24). As explained in Appendix I, this resulted in a decrease of forward light scattering. Also, doses lower than 20 kJ/m² UVR should be employed in further studies, as it is not meaningful to damage the lens that severely.

4.1.2 Possible repair of UVR-induced lens opacities

A skewed distribution of the difference in forward light scattering is found at four weeks and later after exposure to 5 kJ/m² (Appendix I). In Appendix I, it is speculated that 5 kJ/m² UVR may be a threshold dose for UVR-induced cataract. The idea is that a less intense reaction may occur below threshold and a more intense reaction above. The results of the dose-response experiment (Appendix II), however, show that this hypothesis must be discarded. It was found that the intensity of forward light scattering increases continuously with increasing UVR dose. As mentioned above, the skewed distribution did not appear immediately after the UVR exposure, but at four weeks after and later. This may indicate that lens damage increases continuously with increased UVR dose, but can be repaired only if the damage is limited.

As a follow-up of these ideas from Appendix I and II, the morphological development and repair of lens opacities after a dose of 5 kJ/m² was investigated and is described in Appendix VI.

4.1.3 Dose-response after UVR exposure

The intensity of forward light scattering in the lens increases along with UVR dose between 3 and 20 kJ/m². No significant change in intensity of forward light scattering was observed for lower UVR doses (Figure 20)(Appendix II).

The left part of [Figure 20](#) shows the mean difference in forward light scattering between the exposed and non-exposed lens one week after UVR exposure, as a function of the UVR dose applied. For UVR exposures between 0.1 and 14 kJ/m², an exponential function was found which best fit the dose-response data ([Appendix II](#)).

This exponential function probably represents the first part of a sigmoid function, which starts with an exponential increase, curves and flattens asymptotically ([Figure 20](#)). This idea is supported by the earlier findings of Söderberg and Löfgren (1994). They found a logarithmic dependence of forward light scattering on higher UVR doses (up to 300 kJ/m²), which probably represents the second part of the sigmoid function.

Figure 20.

The left part shows the dose-response function for UVR induced cataract (solid line) from experiments using the interference-filter-based source. The right part shows data from experiments using the monochromator-based source. Note that the exposure dose of 8 kJ/m² induces different light scattering, dependent on the exposure source and exposure time.

X-symbols: data from the long-term experiment ([Appendix I](#)), Circles: data from the dose-response experiment ([Appendix II](#))

Open triangle: data from an experiment by Löfgren et al. (1997) and Solid triangles: data from the repeated exposure experiment ([Appendix IV](#)).

The data symbolized by a triangle were obtained simultaneously. Datapoints with open circle and open triangle were obtained with the same dose and same exposure time, but different exposure source. Bars are the 95% confidence interval for the mean difference of forward light scattering between the exposed and non-exposed lens one week after (the second) *in vivo* exposure to UVR.

Sigmoid trendline:

$$v = \frac{1}{1 + e^{3.5524 - 0.2422 \cdot x}}$$

Figure 21.

Intensity of light scattering in representative lens layers as a function of age in humans.

(Reprinted, with permission, from Sasaki (1997). Copyright by Nova Acta Leopoldina, Halle.)

In an epidemiological study, Sasaki (1997) found an exponential increase of lens opacities with increasing age (Figure 21). This exponential increase was found for the entire lens, but was most pronounced for the lens cortex. It is interesting that lens opacities increase exponentially with age as well as with UVR dose. This might support the idea that finite lens damage induced by UVR accumulates over a lifetime and results in cortical cataract.

4.1.4 Macroscopic model for the dose-response function

There may be two types of idealized dose-response functions (Sliney & Wolbarsht 1980b) which both can be modulated by repair. First, there may be a function with a threshold. Here, low doses induce no effect. At a certain dose there is a sharp increase of the dose-response function to the maximal response. Higher doses do not induce more response. Because of variations between individuals this dose-response function can become stochastically modulated. Then the probability for response as a function of dose has a sigmoid curve.

Second, there may be a continuous dose-response relationship. Here, increasing doses induce continuously more severe response. Any single exposure would have a finite effect and a cumulative exposure would increase the response. Then the response as a function of dose is a sigmoid curve.

If repair comes into play, e.g. with a continuous process, then below a certain dose there will be no effect. Above a certain dose, when the injury overwhelms the repair, the dose-response function continues as for the pure continuous process.

The results from Appendix II and the dose-response function in Figure 20 show that very low UVR doses ($< 1.3 \text{ kJ/m}^2$) do not induce increased forward light scattering. For higher doses the dose-response function has a sigmoid shape. This suggests that UVR lens damage is a continuous process with repair according to the above described idealized dose-response functions.

4.1.5 Microscopic model for the dose-response function

One may assume that the crystalline lens is composed of an infinite number of units that may react with a quantal response to UVR, changing from clear to scattering. One may assume also that there is a threshold dose needed to switch from clear to scattering and that it varies randomly among the units.

Considering the central limit theorem*), it is reasonable to believe that the frequency distribution for threshold doses is normally distributed (Figure 22). Then holds, that the proportion of scattering units after a certain dose is described by the integral of the frequency distribution of threshold doses (Figure 22).

If the lens with commencing cataract is simplified to an infinitely dilute solution, the total intensity of light scattering from a number of scattering units is directly proportional to the number of scattering units. Thus, the quantity on the

Figure 22.

Relative frequency (dotted line) and cumulated relative frequency (solid line) of scattering units in the lens as a function of dose of ultraviolet radiation.

right Y-axis in Figure 22 may be changed to total intensity of light scattering in a lens. The sigmoid shape of the cumulated relative frequency is indeed found in the dose-response function for UVR cataract (Figure 20).

4.2 Estimation of a Maximum Acceptable Dose (III)

4.2.1 Setting a lower limit for normal lenses

The level of light scattering of clinically clear lenses varies from individual to individual in accordance with the normal distribution (Söderberg et al. 1990). For this reason, the lower limit or beginning of pathologic light scattering must be defined statistically as the maximal level of light scattering that a certain fraction of normal clinically clear lenses expresses. In Appendix III, this fraction p was set to 97.5 %.

The selection of the fraction of unexposed lenses that shall count as clinically normal influences the Maximum Acceptable Dose (MAD). If a larger fraction of lenses were considered as clinically normal, the resulting MAD would be higher and more severe damage would be accepted. If a smaller fraction of lenses were be considered as clinically normal, the resulting MAD would be lower

and the toxicity estimation would be more conservative, with a lower acceptable dose and therefore less damage. In this way, selecting the limit for normal lenses incorporates the risk which will be accepted with a certain Maximum Acceptable Dose.

4.2.2 Experimental dose-response function

In search of an index for toxicity of UVR on the crystalline lens, only the low-dose-region of the dose-response function is of interest (Figure 20). In Appendix III, the sample size was 20 rats. Therefore, the estimates of the mean and variance of forward light scattering of normal clinically clear lenses have limited precision. With increasing sample size, the estimates would converge towards the expected values.

*) "If random samples of size n are drawn from a normal population, the means of these samples will conform to normal distribution. The distribution of means from a non-normal population will not be normal but will tend towards normality as n increases in size. This result is known as the *central limit theorem*" (Zar 1999a).

4.2.3 Estimation of the Maximum Acceptable Dose

The lower limit or beginning of pathologic light scattering is projected on the dose-response function obtained with the regression analysis of experimental data (Appendix III, Figure 4). The dose corresponding to that point can be estimated and was suggested to be called the Maximum Acceptable Dose (MAD).

4.3 Fractionation of exposure (IV)

4.3.1 Influence of fractionation of exposure

Estimating the biologically effective UVR dose is important for occupational safety standards, evaluating environmental UVR exposure, and in experimental studies. During continuous or intermittent long-term exposure, biological damage can accumulate, be amplified or be repaired; this has to be considered for the above estimation.

UVR doses repeated within different time periods may be said to be additive only if they produce the same effect as the sum of the doses without any interval between them. Duke-Elder and MacFaul (1972) stated that "... the effect of repeated exposures with intermissions is in a general sense equal to their equivalent as a single long exposure, provided that the intervals do not exceed 24 hours ...". Occupational doses are often calculated by adding exposure doses within one day or an 8 h working day. In their guidelines of Threshold Limit Values for chemical substances and physical agents the American Conference of Governmental Industrial Hygienists (ACGIH 1996) considers UVR exposure within an 8 h interval to be additive. One of the few epidemiological studies which estimated the UVR dose over decades (Taylor *et al.* 1988) used a serial additive expected-dose model. In this model (Smith *et al.* 1980), the estimated exposure dose for one day was summed to determine annual exposure.

The experiment described in Appendix IV, fractionated the UVR exposure into two parts with 0, 6, 24 or 48 hours between them. After two UVR exposures, forward light scattering by the rat lens is

The $MAD_{0.975}$ for rat lenses found in this study is of the same order of magnitude as the threshold for permanent cataract in rabbits as reported by Pitts *et al.* (1977). It should, however be kept in mind that the rabbit cornea is thicker than the rat cornea and therefore should filter out more UVR (Table 3 and Figure 11).

the same whether the second UVR exposure is repeated immediately or after a 6 h interval (Figure 20). Therefore, UVR exposure doses applied within a 6 h time period are additive. When the second exposure follows 24 or 48 h after the first, forward light scattering is greater than after the shorter time intervals (Figure 20). UVR doses repeated after 24 or 48 h thus aggravate the injury and are not additive.

Why lens injury is greater when the second UVR dose follows 24 or 48 h after the first is not known. Defense against oxidative stress may be less effective or membrane function might be challenged after a UVR exposure (Hightower 1994, Shindo & Hashimoto 1997). Therefore, a repeated UVR exposure may produce a more severe biological response. Another explanation for the increased susceptibility might be the formation of photoproducts (Hightower 1994) between 24 and 48 h post-exposure. Furthermore, programmed cell death peaks at 24 h after a low-dose-UVR exposure (Appendix V). Replacement of dead cells demands a higher rate of mitosis in the germinative zone and a second UVR exposure at that time may have a more damaging effect.

During the design of the experiment in Appendix IV, we expected a decrease of lens damage with increased time between the exposures. After having performed the experiment, we suspected that the maximum time between exposures (48 h) was too short to allow recovery and repair in the lens. Therefore, a new experiment was designed by Ayala *et al.* (2000) with two exposures separated by 0.25, 1, 3, 9 and 30 days.

4.3.2 Influence of exposure time

Figure 20 includes also one datapoint from an experiment by Löfgren et al. (1997) from a study of the influence of age and gender on the formation of UVR cataract. We used the same dose of 8 kJ/m^2 from the monochromator-based source as in Appendix IV, but delivered during 15 min and not during 30 min as in Appendix IV. The same dose given during half the exposure time induces almost twice the forward light scattering as measured in EDC (Figure 20).

This preliminary result questions the validity of the Bunsen-Roscoe reciprocity law (Bunsen & Roscoe 1862) for UVR-induced forward light scattering. This law says that the amount of chemical change produced is proportional to the amount of light absorbed. In other words, the product of irradiance and time is constant and there is a time reversal symmetry. Even though the Bunsen-Roscoe reciprocity law is a photochemical law and was established based on experiments with photographic paper (Bunsen & Roscoe 1862), it is widely applied in biology. In order to check if the Bunsen-Roscoe reciprocity law holds for UVR induced cataract, Ayala et al. (1999) designed a follow up experiment where the same exposure dose was given during exposure times ranging between 5 and 120 min.

4.3.3 Influence of radiation spectrum

The summary diagram of Figure 20 also draws attention to the fact that the detailed spectral distribution is important for the biological response. The single dose of 8 kJ/m^2 delivered during 15 min induced more than twice the damage when it was given by the monochromator-based source as compared with the interference-filter-based source (Figure 20, open circle and open triangle).

The different spectra for the interference-filter-based and monochromator-based source are given in Figure 23. The corneal dose is the same for both absolute spectra, but the spectrum from the monochromator-based source is more narrow and therefore the radiation dose is more concentrated at 300 nm. The spectral irradiance at 300 nm is about 50% higher behind the monochromator-based source as compared with the interference-filter-based source. Because the wavelength at 300 nm is known to be more damaging to biological tissue, this would explain the greater damage behind the monochromator-based source.

The erythema dose according to the Commission Internationale de l'Eclairage (CIE) is often used to compare the biological effectiveness, not only for erythema. The relative effectiveness for erythema by the CIE is given in Figure 12 and is used for calculation of the corneal dose from both exposure sources. The erythema dose of 5 kJ/m^2 corneal dose is 2.0 kJ/m^2 for the interference-filter-based source and 3.0 kJ/m^2 for the monochromator-based source. This again reveals the 50% more damaging impact of the monochromator-based source.

Figure 23 includes also an estimation of the dose that a rat lens as well as a human lens would receive during the experiments. Transmittance data for the rat and human cornea, as shown in Figure 11, were used for these theoretical estimations. The spectral difference of the radiation reaching the lens from the two sources, becomes even more pronounced after filtering by the cornea.

Figure 23.

Comparison of corneal dose and estimated dose reaching the rat lens and the human lens respectively.

Figure 23a shows this comparison for the interference-filter-based source and Figure 23b for the monochromator-based source.

Data from figure 11 used for estimating the dose reaching the lens.

4.4 Morphological changes and repair (V, VI)

4.4.1 Cornea

Ocular inflammation and corneal damage were recorded subjectively during the long-term experiment (Appendix I) ([Table 4](#)). Observational criteria for “Ocular inflammation” were swollen eyelids, conjunctival injections and discharge. Corneal damage was categorized as superficial opacity, diffuse opacity, micro tumor and neovascularization.

All animal eyes exposed to 20 kJ/m² and 30% of those exposed to 5 kJ/m² went through an inflammation of conjunctiva and cornea ([Table 4](#)). All animal eyes exposed to 20 kJ/m² and 50% of those exposed to 5 kJ/m² got an opaque cornea between 4 and 7 days after exposure. Eyes exposed to the lower dose had less intense inflammation and corneal opacity. An ulcer remained on 50% of the corneas exposed to 20 kJ/m² at 28 days after exposure and thereafter. Blood vessels were observed on 70% of the corneas exposed to 20 kJ/m² and on 30% of those exposed to 5 kJ/m² between 4 and 32 weeks after exposure ([Table 4](#)).

Andley et al. (1996) found a similar time course for corneal and conjunctival damage in the rabbit after UVR exposure. The exposure dose was higher (28 kJ/m²) but the radiation spectrum was more broad than that in the present experiment and had a maximum at 310 nm.

The damage in the cornea was observed between 1 and 3 days and in the conjunctiva between 1 and 4 days after UVR exposure.

4.4.2 Intact lens

One week after exposure to UVR, lens opacities are visible to the naked eye in lenses exposed to 3 kJ/m² or higher doses ([Figure 24](#)). Exposed lenses have haze on their anterior and posterior surface that becomes more intense with increased dose. After exposure to 14 kJ/m², the surface becomes opaque, strong equatorial opacities are visible, and the ciliary body is often attached to the lens. After exposure to 20 kJ/m² the cortical and equatorial opacities are more dense and nuclear opacities could be seen ([Figure 24](#)).

It is stated in the literature that following UVR exposure, “animal opacities mainly occur in the anterior cortical region of the lens whereas human cortical opacities tend to occur in the equatorial (peripheral) parts of the cortical region” (Dolin 1994). This might be the case a short time after experimental exposure to UVR, as seen one week after exposure in the current experiments ([Figure 24](#)).

Corneal dose	5 kJ/m ²	5 kJ/m ²	20 kJ/m ²	20 kJ/m ²
Ocular damage	Animals involved	Duration	Animals involved	Duration
Ocular inflammation	30%	day 4...7	100%	day 3...7
Cornea: superficial opacity	50%	day 4...7	100%	day 4...7
diffuse opacity	no		70%	day 7
micro tumor	no		50%	from 4 weeks on
neovascularization	30%	from 4 weeks on	70%	from 4 weeks on

Table 4.

General summary of ocular damage other than to the lens after UVR exposure; based on miscellaneous observations with the naked eye during the long-term experiment (Appendix I).

Figure 24.

Microphotographs of isolated rat lenses after in vivo exposure to UVR against a black background with a white grid. The distance between the white wires is 0.79 mm. Different UVR doses are presented in columns and different post-exposure times are grouped in rows. The photographs show lenses from different animals.

Post- exposure time (weeks)	Forward light scattering (EDC)					
	non-exposed	3	UVR dose (kJ/m ²)			
			5	8	14	20
1	0.126	0.185	0.311	0.347	0.703	1.030
4	0.110		0.163			0.634
8	no data		no data			
32	no data		no data			

Table 5.

Measured forward light scattering in the lenses shown in figure 24.

After more extended post-exposure intervals (5 kJ/m² after 8 and 32 weeks) or higher doses (8 kJ/m²), opacities are seen mainly in the equatorial region and less in the polar region of the exposed lens. This location of the opacities is more common in human cortical cataract (Figure 24).

The macroscopic appearance of lenses exposed to a high dose of UVR (20 kJ/m²) illustrates the fact that after severe damage, the lens becomes so opaque

that the forward light scattering decreases. This is due to attenuation of transmittance in the lens and is described in Appendix I Figure 24 and Table 5). Foo and Maclean (1996) were confronted with the same effect in human cataract. They mention that severe nuclear cataract can attenuate so much back-scattered light that the lens appears less dense in retroillumination image analysis.

In Appendix VI, the development of lens opacities was studied between 24 h and 8 weeks after a UVR exposure of 5 kJ/m². One day after UVR exposure, lenses had flaked confluent dots or small granules scattered over the anterior surface; the posterior surface looked normal. At 7 days post-exposure, the lens surface was rough and appeared corrugated and equatorial opacities were seen. The anterior side of the lens was more opaque than the posterior. Vacuoles were visible at the lens equator in the cortex. At 56 days post-exposure, the lens surface was clear again. However, a narrow equatorial cortical light scattering ring was visible. Because this light scattering ring was visible in coronal and sagittal view, it was assumed to have a shell shape (Appendix VI, Figure 2).

4.4.3 Lens epithelium

In vivo low dose UVR leads within one day to apoptosis and disintegration of the lens epithelium. After one week the epithelium contains extracellular spaces. Within several weeks after exposure, the lens epithelium recovers (Appendix V and VI).

Necrotic and apoptotic cells have different morphological features: necrotic cells show a dilated endoplasmatic reticulum, swollen mitochondria, ruptured plasma membrane, general cell swelling and groups of cells are affected. Apoptotic cells shrink, lose intercellular contact, undergo fragmentation into membrane bound segments with intact endoplasmatic reticulum, mitochondria, and lysosomes. Apoptosis usually effects individual cells which are then phagocytized by surrounding cells or macrophages (Kuszek 1997).

We consider apoptosis or programmed cell death as a cell response after low dose damage, and necrosis as response after high dose damage. After low dose damage, programmed cell death leaves a greater chance for tissue repair because cells with DNA damage are removed. After high dose damage, the tissue is destroyed by necrosis, with a lower chance for repair.

Andley and co-workers (1999) showed that photoproducts (cyclobutane pyrimidine dimers and (6-4) photoproducts) are eliminated within 1 to 2 days after UVR exposure, depending on UVR dose and type of photoproduct. They conclude that cells either repair DNA damage and proceed in the cell cycle or do not repair it and are eliminated by cell death.

The p53 protein is known to play a role in cell cycle regulation, induction of apoptosis and stabilization of the genome (Wilson 1999). In this way p53 is involved in controlling the cellular response to DNA damaging agents. Cells exposed to UVR raise their concentration of p53 protein. A high level of p53 blocks cell proliferation and prevents the cell from progressing into S phase and replicating DNA. The cells are either delayed in G1 phase or die by programmed cell death (Alberts 1994a).

4.4.4 Lens fibers

One week after low dose UVR exposure, equatorial parts of superficial lens fibers contain extracellular spaces (Appendix VI). The extracellular spaces together with locally disarranged fibers produce a corrugated opaque lens surface and equatorial opacities. Within several weeks after exposure, the lens epithelium recovers, and new fibers develop normally. The lens fibers regain normal water balance and fill up the extracellular spaces. Repair, however, is incomplete, and disarranged fibers remain in the cortex, producing a subtle shell-shaped opacity.

In this way, subtle damage to the lens fibers induced by UVR may accumulate during a lifetime and contribute to the formation of cortical cataract. Indeed, the main type of lens opacification found in the human is cortical cataract (Sasaki 1997) and the rate of opacification in the outer lens cortex is about 4-5 times higher than the nuclear onset of cataracts (Hockwin 1997).

UVR exposure initially causes DNA damage in the lens epithelium which is repaired within a few days, either keeping the cells functioning properly or removing them by programmed cell death (Appendix VI). New epithelial cells proliferate and enable the epithelium to be completely repaired. Damage to the lens fibers is delayed as compared to the epithelium and is restricted to the fibers differentiating at the moment of exposure. How both events, in epithelium and fibers, are linked remains a challenging question. UVR may reach the lens equator by multiple scattering and might damage differentiating fibers directly, or there may be signals from the injured and repairing epithelium which cause the damage of the lens fibers, or the disarranged epithelium may not be able to optimally regulate water and ion homeostasis.

4.5 Reasons for light scattering (VI, VII)

4.5.1 Spatial order of lens fibers

Light is always scattered in the crystalline lens, but a regular spatial order of the lens fibers causes destructive interference of the locally scattered light and hence less total light scattering (Bettelheim 1985, Hemenger 1988). Therefore, the spatial order of the lens fibers is crucial for lens transparency. This fiber order can be evaluated by Fourier image analysis. Before the time of computers which made the calculation of Fourier power spectra very fast, diffraction was used as optical Fourier analysis. Depending on the mathematic algorithm applied, computer Fourier calculations induce errors by rounding and optical Fourier analysis can give more reliable results. The pattern of the diffraction and Fourier peaks from a regular sample corresponds to the geometry and spacing in the sample.

Jose (1986) discussed the signals between the epithelium and the fibers after UVR exposure. If the lens epithelium is disrupted either in morphology or metabolism, its interaction with fibers in the bow may be altered. Also damage to the genome of the epithelial cells may affect the lens fibers, because cues for differentiation that normally pass from the epithelium to the fibers are lost. Consequently, the fibers fail to differentiate normally.

In contrast to epithelial cells, fiber cells do not contain thymidine, which is required for DNA synthesis. Therefore, damage in the fibers cannot be repaired via repair-replication of DNA (Modak 1972).

René Descartes (1596-1650), a French mathematician, scientist, and philosopher was the first to describe a corona due to diffraction by particles in the human eye (Figure 25). The theory of diffraction was not known at that time, but Descartes speculated that the cause of the corona should be in the eye that sees them (Simpson 1953). He published Figure 25 in his "Discours de la méthode" in 1637. In three essays forming part of the Discourse, he illustrated his method for utilizing reason in the search for truth in the sciences. In Dioptrics he then presented the law of refraction, in Meteorology he explained the rainbow, and in Geometry he gave an exposition of analytic geometry ("Descartes, René" Encyclopædia Britannica Online).

Figure 25.

A corona which Descartes observed around the flame of a candle and described in "Discours de la Méthode" (1637).

(Reprinted, with permission, from Simpson (Brit. J. Ophthal. (1953) 37, 450-486). Copyright by BJM Publishing Group, London.)

In a review on ocular halos and coronas, Simpson (1953) explained that Descartes' corona was due to particles on the anterior surface of the cornea. Another diffraction phenomenon seen by the human eye is the lenticular halo. In 1897, Druault explained the lenticular halo due to the circular optical grating formed by the radial fibers at the periphery of the crystalline lens [Figure 2](#) (Druault 1898). In 1953, Simpson estimated the fiber width of his own crystalline lenses from the size of the lenticular halo that he could see (Simpson 1953).

Sir David Brewster was probably the first person who used an optical diffraction technique to estimate the fiber dimensions in animal lenses (Brewster 1816). Philipson (1973) showed a diffraction pattern from slices of cortical areas about 500 μm thick from the human lens illuminated with a helium-neon laser [Figure 26a](#)). The peaks in his diffractogram corresponded to a spacing of 7 μm , most likely caused by the regular spacing of the lens fibers. However, there are only peaks distinguishable in one direction.

Figure 26a.
Diffractogram showing low angle laser light scattering from a section of human transparent lens cortex.
(Reprinted, with permission, from Philipson (1973). Copyright by Academic Press, London.)

This suggests that the lens sample only contained fibers in longitudinal section. A cross-section of the fibers would reveal peaks in two dimensions; fiber width and fiber thickness.

In Appendix VI, Fourier analysis was applied to describe the order of the lens fiber lattice. If there is a regular order in the lattice, the Fourier power spectrum reveals peaks which can be measured and the average fiber dimensions can be estimated. Beyond the scope of Appendix VI, I will give here an idea how changes in the lattice order can be quantitatively described.

From the areas marked in [Figure 27](#), Fourier power spectra were calculated. [Figure 26b](#) gives an example of such a spectrum. Then, intensity profiles were calculated along a line from the center of the Fourier spectrum as indicated by the arrow in [Figure 26b](#). [Figure 28](#) shows these intensity profiles from the areas A, B and C as marked in [Figure 27](#). Each diagram contains the intensity profile from the exposed and non-exposed lens.

Figure 26b.
Example of Fourier power spectrum. Arrow indicates area and direction of intensity profile measured.

Figure 27.
Mid-coronary paraffin lens sections
stained with wheat germ agglutinin.
Areas of the Fourier transform
marked.
(See Appendix VI)

Figure 28.
Intensity profiles of Fourier power spectra
corresponding to the areas marked in figure 27.

All profiles from non-exposed lenses show a peak (first harmonic maximum) which represents the average fiber width (at about 8 μm). High frequencies at the right side of the diagrams contain information about the fiber membrane thickness and the focal quality of the image.

The profiles from the damaged areas of exposed lenses (A and C) do not show clear peaks (Figure 28, Appendix VI, Fig. 5f). Therefore, an average fiber width cannot be estimated. However, a way of comparing the intensity profiles is to compare the areas under the curves (Taylor & Costello 1999). Such a comparison is illustrated by the outlined area between the curves in Figure 28. Quantitative evaluation of the areas between the intensity profiles should consider Fourier frequencies between half and double frequency of the first harmonic maximum.

4.5.2 Refractive index distribution

The lens maintains a gradient of refractive index that allows it to produce an image without significant spherical and chromatical aberration (Clark 1994). In the lens, the refractive index is proportional to the protein concentration. A continuous increase of the protein concentration is established due to regulated protein synthesis and cell dehydration during cell differentiation.

At 6 months after a short-term low dose UVR exposure (5 kJ/m^2), local variations of the refractive index induced a subtle cortical light scattering (Appendix VII, Fig. 8). At the same time, no global change of the refractive index was found between exposed and non-exposed lenses. The local variations in protein density cancel each other out when averaged between the individuals because they are displaced somewhat along the lens radius and they have positive or negative magnitude. The absence of a significant difference of the protein density in the outer cortex, supports the earlier morphological finding (Appendix VI) that the new fibers that grow after the UVR exposure develop normally.

However, lenses exposed to UVR grow slower than their non-exposed contralaterals (Appendix VII, Fig. 6a and 6b). Even though UVR-induced light scattering has almost returned to normal several weeks after low dose exposure, there was a decrease in the mass of these lenses. The finding that both water content and dry mass decreased by about 10% after low dose exposure, shows that the average protein density is kept constant.

This shows how excellent is the capacity of the lens for repair. Despite the fact that the UVR exposure causes a slower growth of the lens, the average protein concentration is kept constant. Also the protein distribution in the fiber layers developed after the exposure recovers to normal. In this way, the complex repair processes maintain the optical function of the lens.

4.5.3 Lens transparency theory

Loss of transparency in a medium can be caused by absorption and scattering. In cataract, absorption plays a minor role (Benedek 1971, Philipson 1969a, b). There are mainly two types of potential scattering centers in the lens: the proteins in the fiber cytoplasm and the fiber membranes. Proteins give rise to small particle scattering ($d < \lambda/5$) and fiber membranes to large particle scattering ($d > 5\lambda$) (Trokkel 1962).

However, the lens is not an opaque tissue, even though proteins and fiber membranes scatter light. Neither, proteins nor fiber membranes scatter independently of their neighbors. Correlation in the position reduces the scattering (Trokkel 1962). In other words, a high degree of spatial order leads to a transparent tissue.

Why high spatial order leads to transparency, can be explained by interference theory (Benedek 1971, Trokkel 1962). Light incident to the lens is reflected and diffracted at proteins and fiber membranes. If proteins and fibers have a *regular spacing*, the reflected or diffracted light is *in phase* and the light waves cancel each other out in directions other than the incident light path (*destructive interference*). As a result, the light is transmitted through the lens along the incident light path.

If protein and fibers have an *irregular spacing* or random variations in their distribution, the reflected or diffracted light is *out of phase* and the waves do not cancel each other out in directions other than the incident light path (*constructive interference*). Behind the lens, this results in light scattering out of the incident light path.

Therefore, *regular spatial fluctuations* decrease light scattering because of destructive interference. Examples for regular spatial fluctuations are the lens fiber lattice in normal lenses (Appendix VI, Fig. 5 and [Figure 27](#)) and the smooth increase of the refractive index in normal lenses (Appendix VII, Fig. 8).

In contrast, *random spatial fluctuations* cause light scattering because of constructive interference. Examples for random spatial fluctuations are the disturbed fiber order in UVR exposed lenses (Appendix VI, Fig. 5 and [Figure 27](#)) and the local variations in the refractive index after exposure (Appendix VII, Fig. 8).

The spatial distribution important for the optical properties of the lens is described by the refractive index. All spatial fluctuation, of proteins or fiber membranes, result in fluctuations of the refractive index. Therefore, the transparency of the lens depends on the smooth and gradual distribution of the refractive index.

5. CONCLUSIONS

- ❑ Building on earlier observations, the current findings indicate that the optimal time to detect low dose UVR-induced cataract is one week after exposure.
- ❑ The dose-response function for UVR-induced cataract is continuous and has a sigmoid shape. The intensity of forward light scattering in the rat lens increases exponentially with increasing UVR dose between 0.1 and 14 kJ/m² and starts to flatten at around 20 kJ/m².
- ❑ A method for experimental estimation of toxicity, for continuous dose response relationships was developed. First, a lower limit of pathological light scattering is defined from the frequency distribution of light scattering of normal clinically clear lenses. Then, the intersection of this limit with the dose-response function gives a dose which is defined as the "maximum acceptable dose" or MAD.
- ❑ Two UVR exposures with a 0 or 6 h interval produce the same degree of lens opacification. When the second exposure follows 24 or 48 h after the first, lenticular damage increases. Repair processes between 24 and 48 h after exposure appear to be sensitive to UVR, and an additional exposure during this time may aggravate cataract development.
- ❑ A UVR dose of twice the maximum acceptable dose induces programmed cell death which peaks 24 h post-exposure and involves the entire epithelium. Dead cells are removed from the epithelium by phagocytosis.
- ❑ A UVR dose of twice the maximum acceptable dose causes cataract which is largely repairable. Extracellular spaces develop in the epithelium and fibers. Within several weeks after exposure, the epithelium recovers and new morphologically normal fibers develop. The originally affected fibers are repaired. However, this repair is incomplete, leaving a small zone of enhanced light scattering in the equatorial cortex.
- ❑ At 6 months after twice the maximum acceptable UVR dose, no global change of the refractive index was found. However, local variations of the refractive index induced a subtle cortical light scattering.

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