29. Light damage to retina and retinal pigment epithelium

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LIGHT AS A MODULATOR AND DAMAGING AGENT IN RETINA AND RETINAL PIGMENT EPITHELIUM

The universal effect of light in the retina is to create the visual signal. Apart from this primary function, however, light can distinctly modify structure and physiology by altering molecular and cellular mechanisms (Remé et al., 1991). Laboratory studies reveal that different ambient illuminances alter the length of rod outer segments, the content of the visual pigment rhodopsin, photoreceptor phospholipid fatty acid composition, antioxidant state (Penn and Anderson, 1991; Penn et al., 1987; 1992; Penn and Williams, 1986) and the levels of key molecules involved in the visual transduction cascade (Farber et al., 1991; Organisciak et al., 1991). Photostasis of the retina is a basic regulative process which ensures that a "set" number of photons per day is absorbed in a given eye. This is achieved by light-dependent and gene-regulated processes (Penn et al., 1986; Schremser and Williams, 1995a; 1995b). Among the physiological systems subject to such regulatory gates are circadian and circannual rhythms, major features in chronobiology (Cahill and Besharse, 1995; Remé et al., 1991).

Apart from such adaptive interactions of light with physiology, ultraviolet (UV) and visible radiation can damage and destroy the retina and pigment epithelium (PE). This apparent paradox has been observed already in ancient history in humans, but systematic research has been incited by Noell's work on light damage in laboratory animals (Noell et al., 1966). Because retina and PE represent a structural unit with functional cross-talk, both will be considered in this article, with the main focus on the PE. There is a variety of extensive reviews on light damage, which will be briefly summarized with their specific emphases outlined.

OVERVIEWS ON RETINAL LIGHT DAMAGE

Comprehensive reviews covering basic mechanisms and clinical aspects including major historical perspectives are given by Lanum, 1978; Organisciak and Winkler, 1994; Sperling, 1980. Discussions on photochemical, photophysical, and general damage mechanisms are found in Andley, 1987; Dillon, 1991; Ham and colleagues, 1984; Handelman and Dratz, 1986; Lawwill, 1982; Williams and Baker, 1980. Action spectra and damage types are analyzed in Kremers and Van Norren, 1988; Zigman, 1993. Prevention of light-induced lesions and possible therapeutic strategies are shown in Gerster, 1991; Tso, 1989. Extensive discussions on instrument hazards are provided in *Ophthalmology*, 1983. Finally, clinical, epidemiological, and age-related aspects are reviewed in Marshall, 1983; 1985; Miller, 1987; Remé and colleagues, 1995a; Terman and colleagues, 1990; Waxler and Hitchins, 1986; Weale, 1989; Young, 1988; 1994.

Studies on human light damage include prospective and retrospective analyses, epidemiological surveys and case reports. The spectral composition of the damaging light source, including sunlight and retinal irradiance levels, varies considerably depending on exposure conditions, the involved individuals, and the methods of analysis. Epidemiological studies are concerned with a potential causal relation of UV and visible radiation to eye diseases such as corneal and conjunctival degenerations, cataracts, retinal and PE degenerative diseases, and retinal aging. There are major discrepancies between epidemiological evidence for such relations and the results obtained in laboratory studies, giving rise to critical views by epidemiologists (Dolin, 1994; Remé and colleagues, 1995a). Irrespective of those discussions, much can be learned from light damage observations in humans, most notably that UV and visible radiation does indeed injure the retina and PE acutely and chronically. Further, human observations may lead the way to the design of controlled laboratory experiments that can avoid the above-mentioned uncertainties and approach basic underlying mechanisms.

Laboratory studies in animals or in vitro systems can control at least some of the confounding factors that complicate human studies. Earlier light damage work in animals displays a broad spectrum of experimental con-

ditions which renders comparisons a difficult task. Nevertheless, fundamental regimens can be distinguished. These include exposure to diffuse, white light, or green light for varying time periods; exposure to constant diffuse, white light, again for varying time periods; exposure to focussed white light or specific wavelengths for short time periods in the hours range; exposure to laser light and to UV light. Whereas the exposure to diffuse, white light may represent the most naturalistic condition, elucidation of action spectra obviously is precluded. An advantage of such regimen is the fact that relatively low light doses can be applied, permitting the analysis of subtle threshold changes on a morphological and biochemical level that may not be readily apparent in funduscopy or electrophysiological testing. Thus, any of these light exposure regimens permits, in its own way, the approach to basic underlying mechanisms, by the analysis of action spectra and potential chromophores, by a dose–response function, by molecular and tissue changes, or by lesion-enhancing or lesion-reducing factors. By far the most frequently applied analytical tool is the evaluation of damage in light and electron microscopic preparations.

Human as well as laboratory studies thus describe light damage as a phenomenon in its own right or as a model system to study retinal and PE diseases including degenerations and dystrophies. As mentioned above, an important aspect perhaps not fully recognized among vision scientists and clinicians is the modulation by light of retinal physiology. This is of particular relevance for circadian rhythm research, because light processed by the visual system represents a crucial zeitgeber signal for the "master clock" in the hypothalamic suprachiasmatic nuclei. Therefore, light that alters the retinal input stage may change the photic signal to the rhythm-generating master clock (Remé et al., 1991). Within a clinical setting one should be aware that diagnostics and therapies involving the exposure to bright light sources may transiently or permanently alter retinal functions. Clinicians investigating retinal physiology and function would be prudent to remember that several basic features can distinctly vary over a 24-hour period as well as within annual seasons (Remé et a., 1991).

LIGHT DAMAGE IN HUMANS

Acute and Chronic Exposure to Sunlight

Several studies report the effects of chronic exposure to sunlight that lead to reduced visual acuity, an elevated threshold of dark adaptation, and reduced night vision (Clark et al., 1946; Hecht et al., 1948; Marlor et al., 1973). A selective loss of blue-cone sensitivity (Werner et al., 1989) and increased incidence of cystic macular edema (Kraff et al., 1985) were found in eyes bearing intraocular lenses without UV filters. An extensive epidemiological survey of watermen in the Chesapeake Bay area concluded that chronic exposure to blue light or visible light, respectively, may be related to the development of age-related macular degeneration, despite the relatively small number of individuals with severe geographic atrophy or disciform scar (Taylor et al., 1992). Acute solar retinopathy is a well-known phenomenon and has been observed in patients after sunbathing, sun gazing, or other outdoor activities (Gladstone and Tasman, 1978; Sadun et al., 1984; Yannuzzi et al., 1987). Of particular interest is the description of histological changes in retina and PE after voluntary sun gazing, because they closely resemble alterations amply documented in animal light damage studies (Hope-Ross et al., 1993).

Acute and Chronic Exposure during Therapeutic Regimens

Chronic cumulative exposure to argon laser blue light reduced the color contrast sensitivity of the treating ophthalmologists (Berninger et al., 1989). Numerous studies describe damage inflicted by ophthalmological instruments, particularly the operating microscope in apparently normal or predisposed eyes (Davidson and Sternberg, 1993; Michels and Sternberg, 1990). Safety recommendations include the use of filters in operating microscopes and the avoidance of coaxial illumination when possible. Endoillumination during vitrectomy may also present a potential hazard (Kuhn et al., 1991). Support for observations in humans comes from studies in monkeys, which were exposed to the light of an operating microscope with lesions in photoreceptors and PE resembling those seen in blue-light injuries (Irvine et al., 1984). Photic maculopathy affecting photoreceptors and PE was induced by an indirect ophthalmoscope (Tso, 1973); repeated exposures to an indirect ophthalmoloscope produced more severe lesions than a single exposure of the same retinal irradiance (Borges et al., 1990).

A recently developed therapeutic strategy prompts attention to retinal and PE safety. Exposure of patients to bright artificial light with illuminance ranges of 2500 to 10,000 lux for up to eight hours is used to treat winter depression (seasonal affective disorder, SAD), circadian sleep-phase disorders, shift-work and jet-lag maladaptation (Terman et al., 1990; Remé et al., 1996). Light regimens may be used for half of the year for periods of decades and more. At present, no ocular lesions have

been observed in patients, but long-term observations are lacking and the question of cumulative subthreshold lesions thus remains (Gallin et al., 1995).

Relevant aspects for all light-induced retinal lesions in humans include predisposing factors such as genetic ones on the one hand and drug-induced photosensitization, eye color, pupil size, or environmental conditions and exposure geometry on the other hand. The latter two conditions are of particular importance and unfortunately often underestimated in epidemiological studies evaluating radiation effects on ocular pathology such as cataracts (Sliney, 1992; 1994).

LIGHT DAMAGE TO RETINA AND PIGMENT EPITHELIUM IN LABORATORY STUDIES

Considering the close anatomical association and functional interdependence of retina and PE it appears problematic to clearly separate light damage in the PE from that in the neural retina in vivo. Only in vitro studies examining the PE in cell culture or the isolated incubated retina may shed light on separate damage mechanisms. A presumed primary lesion in one tissue will lead to responses in the other. For example, a light-induced release of signalling molecules in the retina may alter PE functions such as disk shedding and phagocytosis. (For detailed review, especially on PE lesions, see Waxler et al., 1986.)

Elusive Chromophores and Action Spectra

Retinal and PE light damage is modulated by the absorption and transmission properties of the cornea and mainly, the lens. Therefore, action spectra for retinal and PE light damage will be dependent on those characteristics. For example, human lens absorption significantly changes with age and thus the action spectra in young eyes may be distinctly different from those in older eyes. Similarly, lens transmission varies greatly in animal species: whereas the rodent lens transmits blue and UVA to a high degree (Gorgels and van Norren, 1992), the yellow squirrel lens can act as an efficient UV and blue filter (Collier et al., 1989). This latter observation led to the design of UV- and blue-filtering protective and vision-enhancing spectacle lenses (Zigman, 1990). The UV transmission of rodent lenses is mirrored in photoreceptors that are maxiamlly sensitive to UV (Jacobs et al., 1991).

An *action spectrum* is defined as the light dose that is required to obtain the same biological effect at different wavelengths. There are stringent criteria for elucidating a true action spectrum, which is then called an *analytical action spectrum*. The conditions for an action spectrum to be considered analytical include: the same mechanism and the same quantum yield is present at all wavelengths tested; the absorption spectrum of the chromophore in question is the same in vivo and in vitro; the absorption of inactive chromophores and light scattering is negligible. Finally, not more than half of the incident quanta should be absorbed by the sample in the wavelength range of interest and the effect must be the same regardless of the rate at which the light is provided, that is, the effect should not change whether a given light dose is applied in a short time or over a longer time period. By contrast, a higher level of complexity is encountered in multicellular systems which restrain the elucidation of individual chromophores and yield polychromatic action spectra (Coohill, 1992; Grossweiner, 1989). In practice, most light damage studies have been done in a way that precludes an analytical action spectrum. For the retina, the visual pigments are primary candidates for triggering light damage. However, numerous studies reveal that some types of light damage may be potentiated and perhaps in some cases initiated by several other chromophores (see below).

Visual pigments. Visual pigments as chromophores for retinal light damage may present a confusing enigma for physiologists and clincians. Why would the visual cells that are exquisitely designed for photon absorption be damaged by light? In humans, there may be a variety of damage-promoting conditions either endogenously present or induced by therapeutic or other manipulations (see below). Animal models or in vitro preparations, as many other model systems, may exaggerate their variables (such as the light dose) in order to obtain unequivocal effects. Changes observed in such "exaggerated" models may be qualitatively similar in the human eye; they may occur, however, over extended time periods at low levels and gradually develop into manifest lesions corresponding to those observed in animal models. Thus, such model systems are indispensable tools to unravel pathogenetic mechanisms in humans.

Using diffuse green light of varying intensities, W. Noell distinguished type I and type II damage. Type I lesions were found in retina and PE after short exposures to high light levels, whereas type II lesions occured after extended exposures to low illuminances. Both were rhodopsin mediated (Noell et al., 1966). The work of van Norren and colleagues distinguishes class I and class II lesions. Class I is created by diffuse visible light applied in low doses and is probably rhodopsin mediated, class II injuries peak in the UVA and blue wavelengths range (Gorgels and van Norren, 1995; Kremers et al.,

1988; Kremers and van Norren, 1989—for extensive review and discussion the reader is referred to Organisciak et al., 1994).

Diffuse green or white light at relatively low doses used for extended time periods in various rodent models is one of the most frequently applied paradigms and is characterized in detail by Organisciak and colleagues (1994). The studies of T. P. Williams and his colleagues contributed classical concepts for this model system and developed the principle of photostasis (Penn et al., 1986) that may not be limited to the albino rat model but may gain a broad significance for several biological systems such as invertebrates and plants (T. P. Williams, personal communication). In the rodent retina, cones appear to be less susceptible to damage than the predominant rods (LaVail, 1976) and genetic regulation determines the extent of damage (LaVail and Gorrin, 1987). Diffuse green light is used in the analysis of damage mechanisms and damage prevention (Fu et al., 1992; Li et al., 1993; Organisciak et al., 1994).

Light-induced lesions to cones were investigated in primates, with blue cones being selectively damaged by intermittent, focused, narrow-band blue light. By contrast, damage to PE prevailed when the light was applied continuously (Kalloniatis and Harwerth, 1993; Sperling, 1980; Sperling et al., 1980). Pigeon cones but not rods were damaged by diffuse light of 3000 nits applied for 6–48 hours (Marshall et al., 1972). Similarly, cone thresholds were lower in monkeys for exposure to diffuse white light, as evaluated by light and electron microscopy (Sykes et al., 1981). In the rabbit retina, rods, cones, and the pigment epithelium were injured by focussed blue-green light as assessed by light and electron microscopy (Hoppeler et al., 1988).

The blue-light mystery. Whereas earlier studies had appeared to limit blue-light-induced lesions exclusively to the primate retina, more recent developments clearly showed similar mechanisms in rodents and other species (van Norren and Schellekens, 1990). Apart from blue-cone lesions, the hazard presented by laser light in earlier studies was thought to affect mainly the PE photochemically—and, at longer wavelengths, thermally (Ham and Mueller, 1976; Ham et al., 1978; 1979). Mediators of blue-light lesions other than the visual pigments may include chromophores which may reside in the PE, in photoreceptors and perhaps in other retinal layers. To date, no key chromophores directly linked to blue-light lesions have been identified, however. On the other hand, there are numerous studies providing indirect evidence for the existence of molecules that could mediate blue- and UV-induced lesions, respectively. Such chromophores may also act as "adjuvants", potentiating rather than initiating the lesions.

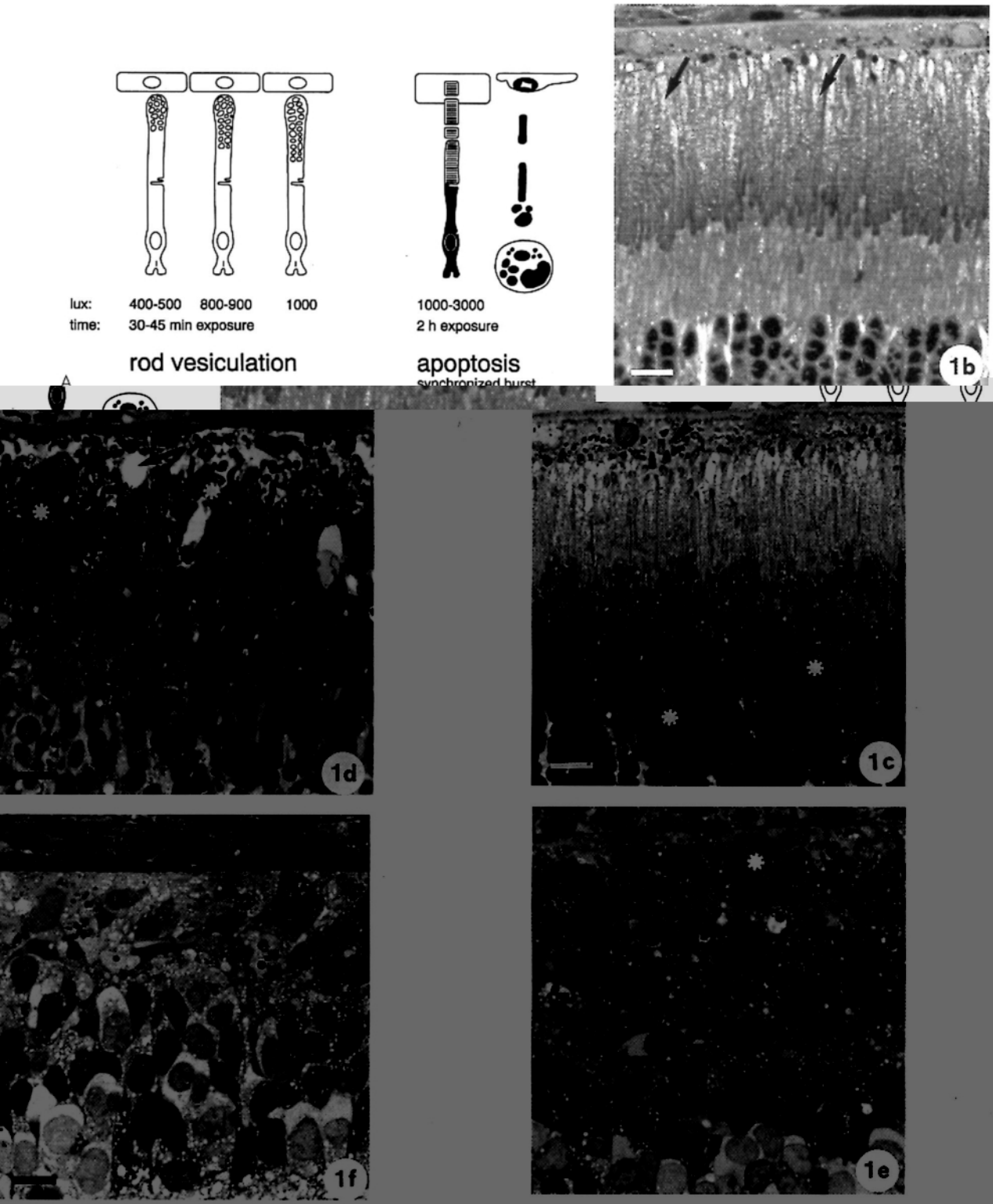
In their study using cultured bovine PE exposed to 435 nm light, Crockett and Lawwill (1984) suggested several chromophores in the PE that could mediate oxygen-dependent photodynamic reactions, such as amino acids, flavins and hemoproteins. In the isolated bovine pigment epithelium, exposure to blue light reduced the transepithelial potential and the short circuit current and induced morphological changes in mitochondria. The action spectrum of changes closely matched that of the respiratory mitochondrial enzyme cytochrome oxidase c and possibly other hemoproteins (Pautler et al., 1990). Exposure of rats to focused narrow-band blue light in vivo inhibited cytochrome oxidase in retina and PE and led to retinal damage as quantified by morphometry, histochemistry, and microradiography (Chen, 1993). A photosensitivity with a peak at 520 nm of the isolated bovine PE was found, resulting in the release of arachidonic acid and increasing the ethanol-induced transepithelial response (Pautler, 1994). Similarly, irradiation of pigment epithelial cells in culture with near UV caused severe damage that was reduced by the addition of catalase (Liu et al., 1995). Light damage was also observed in temperature-controlled pigment epithelium cultures upon irradiation with different wavelengths (Olsen et al., 1995). Narrow-band blue light of 439 nm was most effective in the induction of blood–retina-barrier-dysfunction as assessed morphologically and with fluorometry with no differences in pigmented versus albino rabbits (Putting et al., 1994). Focused UVA and blue-light-inflicted lesions in the rat retina similar to those noted in earlier work as assessed by funduscopy (van Norren et al., 1990). When the effects of collimated UVA light and green light were compared by light and electron microscopy, a remarkable similarity of lesions emerged, indicating the possibility of common underlying mechanisms (Rapp and Smith, 1992b). However, UVA light was more effective in causing photoreceptor cell death. Furthermore, the synthesis of new rod outer segment disks was slowed after UV exposure (Rapp et al., 1994). Both the retina and PE were heavily injured after exposure to collimated monochromatic UV light of 366 nm in aphakic gray squirrels, whereas phakic animals remained uninjured due to the UV-absorbing property of the squirrel lens (Collier et al., 1989). The chromophore for lesions induced exclusively by UV without visible light remains to be elucidated, however.

Recent studies in our laboratory indicate that monochromatic blue light (403 nm, 10nm bandwidth) can induce the so called photoreversal of rhodopsin bleaching

in vivo. After bleaching of rhodopsin by intense green light (550 nm, 10 nm bandwidth, 47mW/cm^2) followed by blue light (403nm, 10 nm bandwidth, 33mW/cm^2) about 30% of rhodopsin was regenerated in the living animal. The evaluation of retinal morphology in animals exposed under the same conditions revealed no damage after green light exposure, whereas massive apoptotic cell death was seen in retinas exposed to blue. The photoreversal has long been known to occur in vitro, whereas photoreversal in vivo has not been shown previously. Our data thus suggest that a long-lived blue absorbing photoproduct is generated from rhodopsin, and this absorber photoregulates rhodopsin when it absorbs blue light (Remé et al., 1998; Williams et al., 1998). We conclude that either rhodopsin itself or a blue-absorbing photoproduct induces the massive apoptotic cell death observed after exposure to blue. Green light, which did not cause apoptosis, was unable to photoregenerate rhodopsin and did not appear to react with any photoproduct.

Pigment epithelial chromophores as candidates involved in light damage. In recent studies, attention has been focused on components in PE lipofuscin as potential chromophores that may participate in initiating or promoting light damage. Lipofuscin has long been suspected to contribute significantly to retinal aging and to age-related macular degeneration. It was assumed that the granule burden in PE cells would partially or totally hamper important PE functions. It remained unclear, however, how PE and photoreceptor cell death was brought about and how the well-known alterations of Bruch's membrane occurred. Photophysical studies on purified intact human lipofuscin granules from different age groups revealed a distinct increase in fluorescence with age and demonstrated three different fluorophores emitting in the blue, yellow, and orange ranges (Docchio et al., 1991). Furthermore, a wavelength-dependent oxygen uptake of PE cells with the generation of singlet oxygen, superoxide anion, hydrogen peroxide, and enhanced lipid peroxidation was shown (Rozanowska et al., 1995). Time-resolved experiments monitored fluorescence decay, UV-visible absorption of longer-lived excited states, and the formation and decay of singlet oxygen in extracts from human lipofuscin, synthetic lipofuscin, and a synthetic orange-emitting fluorophore. The experiments demonstrated that all three compounds absorb in the UV and visible range and can act as sensitizers for creating reactive oxygen species (a triplet state, a radical, singlet oxygen). They may thus be involved in age-related cell loss and degeneration including apoptotic cell death and their relation to light exposure in that they can potentiate light-induced lesions (Gaillard et al., 1995). Those studies shed new light on mechanisms of retinal and PE aging and degenerative changes. Because lipofuscin occurs in vast amounts and accumulates already in young eyes, its sensitizing action might represent a property fundamental to human disease. In vivo fluorescence measurements in human eyes confirm the spectral characteristics of lipofuscin and may thus represent a valuable diagnostic and prognostic tool for evaluation of age-related and other degenerative changes (Delori et al., 1995). Lipids in human lipofuscin fractions, compared with those from rod outer segments, show a different composition that is more pronounced with age, supporting the concept that lipofuscin does not merely reflect rod outer segments but is the result of complex chemical reactions (Bazan et al., 1990). Recent studies demonstrated a blue- and UV-absorbing opsin in the PE. This opsin is distinct from rhodopsin and cone visual pigments, contains the all-*trans*-retinal Schiff base and has absorption maxima at 469 and 370 nm (Hao and Fong, 1996). Those molecules may thus represent further candidates for promoting blue-light lesions. An important autofluorescent component of PE lipofuscin was recently isolated, purified, and characterized. It is a pyridinium bis-retinoid (N-retinylidene-N-retinyletha-nolamine, A2-E) that exhibits detergent properties and inhibits lysosomal functions (Eldred and Lasky, 1993; Kopitz et al., 1996). Due to its properties, it may contribute to changes leading to PE diseases such as age related macular degeneration. In addition, it may act as a chromophore, absorbing light in the UV and visible part of the spectrum. Our laboratory developed a specific and sensitive quantitative assay to monitor this compound, which was found to increase significantly with age in the rat (Reinboth et al., 1997).

PE melanin has long been claimed as a chromophore for blue-light damage because it absorbs exponentially more in the blue and UV spectral range (Ham et al., 1986). By the same mechanism it was suggested that PE melanin protects against damage (Sanyal and Zeilmaker, 1988). However, other studies could not confirm this protective role but rather found no difference in the amount of light damage in pigmented versus albino eyes (Hoppeler et al., 1988; LaVail et al., 1987; Putting et al., 1994; Rapp and Smith, 1992a) or in heavily pigmented fundus areas as compared to lightly pigmented ones (Howell et al., 1982; Lawwill, 1973). When exposure to light in albino and pigmented rats was equated in terms of its effectiveness to bleach rhodopsin, damage was equal in the two strains of rats (Rapp and Williams, 1980). Notably, the pigmentation of the iris may well protect against incident light and thus act in a protec-



er exposure (>24 hours). Exposure to 3000 lux for 2 hours qualitatively identical changes as observed after 1000 lux for however the lesions are now spread over the entire ocular xcept the far periphery.
roscopic pictures illustrating ROS lesions as well as the turn- with apoptotic lesions and scar formation in the albino rat) ROS alterations (↑) seen immediately after exposure to

FIGURE 29-1. (a)Schematic drawing indicating photoreceptor changes as a function of illuminance duration and intensity, illustrated for the albino rat retina. Lesions are confined to rod outer segments (ROS) after exposures of 500–1000 lux for 30–45 minutes. Exposure to 1000 lux for 2 hours represents a turning point, with apoptotic cell death occuring in the lower central retina, followed by massive cellular decay, apoptotic bodies and macrophage invasion at later time

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tive manner. In light of those studies, melanin might be considered an ambivalent compound with potentially protective, damage enhancing, or neutral qualities. Similar observations were made in the skin and expressed in the following way: "Is melanin photoprotective? Sometimes yes, sometimes no." (Giacomoni, 1995).

Exogenous photosensitizing chromophores. There is a vast amount of drugs which potentially could act as photosensitizer for retina and PE, provided that they pass the blood–retina interface, absorb in the near UV and visible range, and either have a cationic-amphiphilic nature, show a porphyrin-like structure, or show a tricyclic-heterocyclic ring system. A number of them have been shown relevant for the eye (Roberts et al., 1992). Clinical observations diagnosed a pigment retinopathy in patients treated with the antiarrhythmic amiodarone. Subsequent laboratory studies revealed a photosensitization of PE cells with increased cell death after irradiation with visible blue and UV light (Dinda et al., 1992; Minelli et al., 1991). The well-known sensitizer phenothiazine can also affect the pigment epithelium (Fox et al., 1993), as can the diuretic hydrochlorothiazide (Hartzer et al., 1993). Rose bengal, a strong sensitizer and closely related to fluorescein, which is known to every ophthalmologist for fluorescence angiography, was found to sensitize the PE by forming reactive oxygen species (Menon et al., 1992). Therapeutic strategies using various porphyrins span a wide area of clinical and laboratory investigations (Gomer, 1991) and porphyrins are suggested to contribute to hematogenous photosensitization of the outer retina (Gottsch et al., 1990). The addition of protoporphyrin IX to pigment epithelium in culture that was irradiated with blue light caused an increase in light damage, supporting the idea that hematogenous photosensitization may occur under certain circumstances (Bynoe et al., 1995). Investigators using the rodent model for light damage studies should remember the Harderian gland, located at the posterior pole of the eye in the orbit, which is a source of a number of different porphyrins (Shirama and Hokano, 1991) and may thus sensitize retina and PE. The importance of photosensitizing drugs should be borne in mind by various clinical disciplines such as ophthalmology and dermatology, but also psychiatry, internal medicine, and others.

Different Light Exposures Answer Different Questions

White light or broad spectral ranges can be applied as collimated beam or as diffuse radiation. Light focussed on the retina will mostly cover a small area and is applied in a high dose within relatively short time periods to anesthetized animals with dilated pupils. Those regimens often mimick instrument hazards such as the operating microscope or the indirect ophthalmoscope. Diffuse white or green light is used for extended time periods or constantly over days to weeks; light doses vary per experiment and, obviously, with the time period of application. The animals are not anesthetized. Quantitative analyses frequently measure the end stage of injury, namely, the reduction or loss of the outer nuclear layer of the retina. This type of paradigm crudely mimicks outdoor exposure (without exactly paralleling a given solar spectrum).

Our laboratory developed a rat model where low light doses are applied for short time periods (Fig. 29-1a). Initial changes are confined to rod outer segments and are reversible within one week. At higher doses, apoptotic death of single cells is observed in photoreceptors and PE, whereas at still higher illuminances, massive apoptosis leading to large areas of decay prevails in the acute stage, followed by a marked macrophage response, proliferative changes and scar formation replacing photoreceptors and PE (Hafezi et al., 1997a; Szczesny et al., 1995). This model does not allow the definition of action spectra, but it permits a detailed analysis of the threshold for individual changes and the determination of their time course in the range of minutes, hours, and days (Fig. 29-1b–f).

Extended exposure durations in any of the above regimens will allow a host of secondary changes to occur and may thus obscure primary events for analysis. For example, it may be impossible to determine whether the PE or the retina is initially and mainly affected by a given light exposure.

500–1000 lux for 30–45 minutes. Lesions are reversible within one week. (c) Changes observed immediately after exposure to 3000 lux for 2 hours. Nuclear (*) and cytoplasmic (↑) condensations indicating apoptosis are seen in photoreceptors. Note the abundance of newly shed phagosomes in the PE (∇), indicating that a shedding burst can be elicited by bright light irrespective of the circadian disk-shedding rhythm. (d) 36–48 hours after exposure to 3000 lux for 2 hours, edema (↑) and macrophages (*) are seen in the area of ROS, PE cells show apoptotic condensation of nuclei (∇) and cytoplasm, and numerous apoptotic bodies (↑↑) appear in the outer nuclear layer. (e) 72 hours after exposure to 3000 lux for 2 hours, most of the photoreceptor nuclei and the PE have vanished. Large macrophages (*), mitotic figures (↑), and abundant cellular debris are seen. (f) 6 days after exposure to 3000 lux for 2 hours, most of the debris has been removed; the choroid shows a dense network of cells and fibers (↑). Some macrophages and glia cells appear in the region of the outer retina (*). Bar represents 10 μm.

Notably, not even diffuse white light creates uniform changes in the retina or PE despite a uniform rhodopsin bleaching (Williams and Webbers, 1995). The work of T. P. Williams and colleagues analyzed in detail the classical sensitive area in the upper temporal region of the rat retina that differs in biochemical and structural parameters from the remaining fundus and is most affected after diffuse white-light exposure (Rapp et al., 1980). In our rat light-damage model, where the light source is mounted above the exposure chambers and shielded by a diffusing screen, the lower central retina shows stronger responses than the other parts at threshold illuminances and short exposure durations (30 minutes–2 hours). In light-damage studies in rats with a transsection of the optic nerve, it is suggested that dopaminergic neurons in the sensitive area may exert protective effects (Bush and Williams, 1991).

Methods of Damage Evaluation

Qualitative and quantitative estimates of lesions span a wide range including psychophysical and electrophysiological methods, fundus reflectometry and spectrophotometry, funduscopy, light and electron microscopy, and biochemistry. The choice of method will depend on the given clinical or experimental situation. It is likely, though, that certain experimental paradigms such as the damage threshold and magnitude, the time course of lesion, and the qualitiy of a change may depend on the method of evaluation. Among other reasons this renders comparisons of data between laboratories rather complex. When large fields of monkey retinas were illuminated by broad-spectrum fluorescent light for 12 hours, the threshold for cones was 6000 to 11,000 lux and for rods 11,000–19,000 lux with histological evaluation 15 hours after exposure (Sykes et al., 1981). The exposure of small patches of monkey retinas under Maxwellian view with a xenon arc light source resulted in a threshold irradiant dose of 230 J/cm^2 for exposures from 10 minutes to 12 hours. The evaluation was by funduscopy and densitometry. Distinct funduscopically visible lesions occured two days after exposure (Kremers et al., 1989). Kremers and van Norren calculated the threshold irradiant dose in Sykes's experiments to be 16 J/cm^2. The distinctly lower threshold in Sykes's experiments may be due, at least in part, to the method of evaluation: light and electron microscopy is likely to reveal subtle changes that will remain unnoticed with funduscopy. Furthermore, a distinction between rod and cone lesions is possible. In addition, the timing of evaluation may be crucial. Whereas funduscopic lesions are clearest 2 days after exposure, the morphological changes are seen already 15 hours following the retinal illumination. In our rat model, apoptotic cell death occurs in photoreceptors immediately after light exposure, whereas the pigment epithelium consistently shows a time lag of at least 5 hours (Hafezi et al., 1997a). An analysis of only early postexposure time points would find the pigment epithelium uninjured. By contrast, evaluation time points later than 24 hours after exposure reveal lesions of both photoreceptors and pigment epithelium with massive cell death and macrophage responses. Thus, the quality of a change may vary as distinctly as the threshold, depending on the method and the timing of an analysis.

MOLECULAR, CELLULAR, AND TISSUE RESPONSES IN LIGHT DAMAGE

In the human eye, damage mechanisms obviously cannot be evaluated as extensively and systematically as in laboratory studies. Clinically, functional and funduscopic changes of the acute solar retinopathy are reported, whereas photochemical and biochemical data are lacking. There are reports, however, on histological changes after sunlight exposure (Hope-Ross et al., 1993).

Molecular Mechanisms in Light Damage

Initial light damage mechanisms on a molecular level still remain unclear, even though there is a large number of studies devoted to different aspects of light-induced lesions (reviewed in detail in Organisciak et al., 1994). Photochemical events may include the formation of singlet oxygen, hydroxyl radical, hydrogen peroxide, and other toxic photoproducts as shown by the experimental application of various antioxidants that reduce light damage (Dillon, 1991; Organisciak et al., 1994). In those studies, the protective agents served as experimental tools that suggested underlying processes. Of special interest is the role of ascorbate that may predominantly protect the pigment epithelium (Organisciak et al., 1994).

Hemoxygenase is the rate-limiting enzyme in heme degradation and is induced in many cell types by oxidative stress (Stocker, 1990). Hemoxygenase I was induced by exposure to intense green light in the rat retina, and this effect was suppressed by the antioxidant dimethylurea (Kutty et al., 1995). When human pigment epithelial cells in culture were transfected with adenovirus-hemoxygenase 1-construct, the cells overexpressed human hemoxygenase 1 and were protected against the toxicity of heme/hemoglobin that was added to the culture (Dunn et al., 1995). Notably, the cytokine

transforming growth factor-β induced hemoxygenase 1 in human pigment epithelial cells (Kutty et al., 1994). The mechanisms involved in the protective effects of growth factors on chronic light-induced lesions are unknown (Collins et al., 1994; Faktorovich et al., 1992; LaVail et al., 1992). Conceivably, the suppression of apoptotic cell death may be involved (Collins et al., 1994). An elevated level of the protein clusterin was observed in light-induced lesions in the rat retina and this increase was reduced by the anitoxidant dimethylurea (Wong et al., 1995a). Increased clusterin levels are also associated with apoptotic cell death in the *rd* mouse model of human retinitis pigmentosa (Wong, 1994).

Particular attention has been paid to the role of lipids and lipid peroxydation in retinal and pigment epithelial light damage (Organisciak et al., 1994; Penn et al., 1991; Wiegand et al., 1983). The retina normally contains more than 60 mol% of polyunsaturated fatty acids, the most abundant ones are arachidonic acid (AA; 20:4 n-6) and docosahexaenoic acid (DHA; 22:6 n-3) (Fliesler and Anderson, 1983). Dietary manipulation of fatty acids, particularly DHA, altered the susceptibility to light-induced lesions. When retinal DHA was lowered (Organisciak et al., 1987) or practically absent (Bush et al., 1991), light damage was significantly reduced or absent, respectively. Paradoxically, in animals with significantly reduced retinal DHA levels showing no acute light damage, the rhodopsin content was distinctly increased. In those animals, however, the rhodopsin regeneration rate was slowed and the photon catch during light exposure reduced to half of controls, implicating a reduced retinal light sensitivity and possibly a role of DHA in normal pigment epithelial physiologic phenomena, such as the visual cycle, shedding, and phagocytosis or fatty acid esterification (Bush et al., 1994). No increase in acute retinal and PE light damage and virtually no peroxidized lipids in vivo were observed in rats fed a diet enriched in DHA and its precursor eicosapentaenoic acid (EPA; 20:5 n-3). However, the ratio of EPA to AA was increased in fish-oil-fed rats, indicating that AA, the precursor of potent and harmful inflammatory mediators, was reduced. Furthermore, a moderately protective effect in fish-oil-fed rats was observed in quantitative analysis (Remé et al., 1994). This apparently contradictory observation supports the idea that mechanisms other than lipid peroxidation may be the primary events in light damage. In a series of chronic experiments, rats fed a diet rich in n-3 fatty acids were more susceptible to a light exposure of 24 hours followed by 10 days of dim cyclic light (Koutz et al., 1995; Wiegand et al., 1995). These observations may indicate that lipid peroxidation occurs within longer experimental periods, perhaps secondary to other, initial events.

Fatty Acid Metabolites as Inflammatory Mediators and Cellular Signaling Molecules

Histological evaluation in numerous light damage studies reveals subacute and chronic changes that show some characteristics of an inflammatory response, such as edema, cell death, cell proliferation, and presence of mononuclear cells (Hoppeler et al., 1988; O'Steen and Karcioglu, 1974; Tso, 1973). The mediators of such changes are not known in detail. AA is the precursor molecule of a variety of inflammatory mediators (Samuelsson, 1991) and is also directly involved in cellular signaling (Axelrod et al., 1988). Furthermore, lipoxygenase products of AA play a role in neuronal transmembrane signaling (Piomelli and Greengard, 1990). The retinal phospholipids are rich in AA and thus furnish ample substrate molecules for cyclooxygenase and lipoxygenase, the major metabolizing enzymes that produce the eicosanoids. A light-induced release of AA was shown in the isolated rat retina (Jung and Remé, 1994) and isolated rod outer segments (Birkle and Bazan, 1989). Recent studies also demonstrate a light-induced release of the lipoxygenase product leukotriene B_4 (LTB_4) in vitro (Reinboth et al., 1995). The releases of both AA and LTB_4 were intensity and time dependent and were inhibited in part by the phospholipase A_2 inhibitor quinacrine and the lipoxygenase inhibitor zileuton, respectively, suggesting finely tuned light-regulated release mechanisms (Jung et al., 1994; Reinboth et al., 1995). In addition, DHA is released by light exposure with kinetics similar to that of AA, implying a further source of inflammatory mediators, albeit less potent, the docosanoids (Reinboth et al., 1996). It is tempting to speculate that there is a functional interaction between AA and DHA release mechanisms. Indeed, preliminary data show an inhibition of AA release by free DHA or free AA in the retina vitro, suggesting the existence of an interaction of free PUFAs with phospholipases as observed in other systems (Reinboth et al., in preparation).

The observations on light-evoked release of lipid mediators led us to propose a concept for retinal light damage mechanisms and possibly other ocular disease processes (Fig. 29-2). The light-released fatty acids and their enzymatically peroxidized metabolites subserve messenger functions and mediate inflammatory and immune responses. Once triggered by light, the mediators may stimulate the PE (Jaffe et al., 1995) or retinal Müller cells (Drescher and Whittum-Hudson, 1996) to release cytokines. Furthermore, the invasion of mononuclear cells is initiated, as documented in histological sections of different light damage models. The mononuclear cells themselves may also release cy-

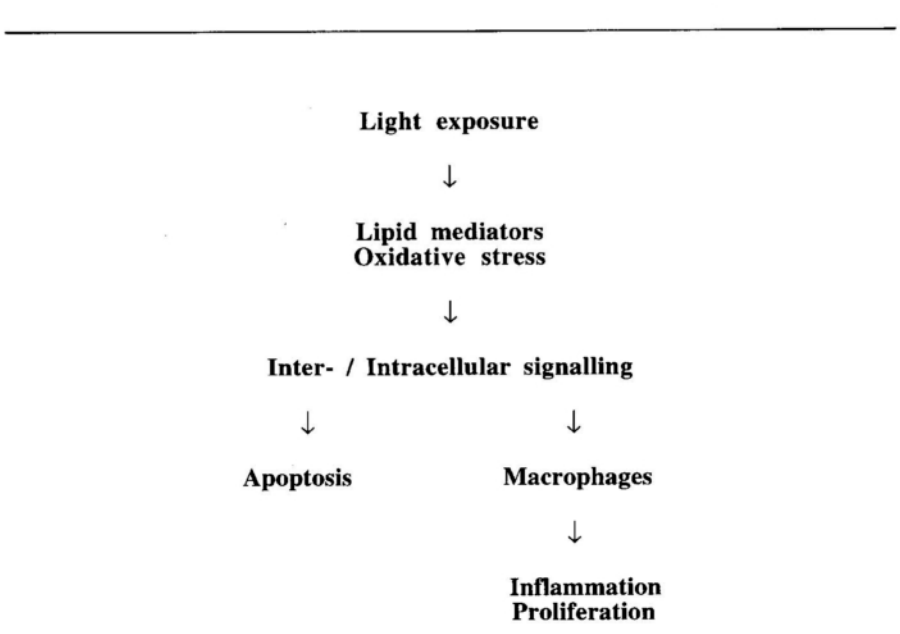

FIGURE 29-2. Cascade of events that could lead to different cellular responses after light exposure. Lipid mediators and molecules of oxidative stress arising after light exposure can initiate different intracellular signaling pathways (such as the release of certain cytokines) that may activate effectors of apoptosis. Lipid mediators are also messengers for the induction of macrophage responses and/or the release of cytokines, which may then lead to inflammatory and proliferative tissue reactions. The conditions that could lead to one or the other of the above described cascades remain to be elucidated.

tokines, which could sustain a cascade of different cellular reactions (Planck et al., 1993; Rappolee and Werb, 1992; Rosenbaum, 1993; Rosenbaum et al., 1987; Wiedemann, 1992). For example, the cytokine interleukin-1β is released by monocytes/macrophages. It can induce the hydrolysis of phospholipids with production of diacylglycerol (Rosoff et al., 1988), protein phosphorylation and cellular signaling via G-protein coupling or transcription factor phosphorylation in various tissues (Dinarello, 1994), and an inflammatory response in ocular tissues (Claudio et al., 1994; Kulkarni and Mancino, 1993; Martiney et al., 1992) as well as cytokine production (Yarosh, 1994) in the skin, indicating the potential for similar responses to radiation in both the eye and the skin.

Vascular endothelial growth factor (VEGF) gene expression is induced in the retinal pigment epithelium (Shima et al., 1995) and other retinal cells (Pe'er et al., 1995; Pierce et al., 1995) by hypoxia in vivo and in vitro. Pigment epithelial cells in vitro stimulate vessel formation from choroidal endothelial cells, an effect that is inhibited by antibodies against various cytokines including VEGF (Sakamoto et al., 1995). In the developing retina, VEGF is expressed by glia cells at certain stages, possibly induced by tissue hypoxia preceding vessel formation (Hata et al., 1995; Stone et al., 1995). Because molecular and cellular responses in hypoxia/ischemia and light damage show striking similarities—such as the hydrolysis and the metabolization of phospholipids, altered levels of intracellular calcium, and proliferative changes—it is tempting to assume that in chronic light damage, too, VEGF gene expression is induced, perhaps mediated by cytokines. IL-1 gene expression was indeed observed in retinal ischemia (Hangai et al., 1995). Furthermore, growth factors provide protection in retinal ischemia (Unoki and LaVail, 1994; Zhang et al., 1994) similar to light damage (Faktorovich et al., 1992; LaVail et al., 1992). The cellular mechanisms by which growth factors can rescue the retina and photoreceptors are unclear to date. It is well known, however, that individual as well as groups of cytokines can have a variety of different effects (Dinarello, 1994) consistent with their potential involvement in light damage as promoters as well as protectors.

In view of dietary modification of retinal light damage susceptibility, it is important to note that supplementation with n-3 long-chain fatty acids like DHA, EPA, or their precursors, suppress the formation of

some cytokines such as IL-1 and tumor necrosis factor (Endres et al., 1989; Meydani et al., 1993). It remains to be seen in animal models of light damage or in human ocular pathology whether n-3 fatty acid supplementation can influence disease processes. The importance of n-3 fatty acids for retinal and brain development and function has been repeatedly demonstrated (Hoffmann et al., 1993; Neuringer et al., 1986). The dietary supplementation with n-3 long-chain fatty acids for inflammatory and immune responses in eye diseases may represent an important therapeutic strategy in the future. Recent data from our laboratory showing that DHA can inhibit the release of AA in the light-exposed retina in vitro supports this notion (Reinboth et al., in preparation). With the suppression of AA release the precursor molecule for potent inflammatory and immune mediators would be eliminated or reduced, respectively, and replaced by the precursor of less potent messengers.

Apart from the induction of cytokine responses by lipid mediators, apoptotic cell death also could be incurred. Lipid mediators and some of their intermediates as well as molecules of oxidative stress (such as NO) were observed to induce apoptosis in nonocular tissues (Buttke and Sandstrom, 1994) (see "Potential Gene Expression . . ." below). The intracellular pathways leading to the execution of apoptosis may include signaling by cytokines via membrane receptors (Barinaga, 1996; Martin and Green, 1995).

In the context of retinal light damage, both of the hypothetical cascades may occur after the light-induced release of lipid mediators. It remains to be seen which conditions would lead toward the apoptosis cascade and which toward the chronic proliferative changes.

Modes of Cell Death: Apoptosis and Necrosis

Cell death by means of apoptosis is of outstanding interest for scientists in a large variety of research fields including cancer research, immunology, virology, degenerative diseases, and radiobiology. The unique mode of this cell death appears to stimulate the imagination as illustrated in phrases such as "death by informed consent" (Gregory and Bird, 1995), "death at an early age" (Papermaster and Windle, 1995), "no self-respecting cell would be seen dead other than by apoptosis these days" (Allen and Goldberg, 1995), "cellular suicide," "altruistic cell death," and others.

The term "programmed cell death" (PCD) was originally used in development to describe a tightly regulated process in organ and tissue remodeling in response to physiological stimuli, requiring de novo gene expression. PCD is not identical to apoptosis, nevertheless the terms are often used interchangeably. Most apoptotic phenomena require de novo gene expression (Schwartz and Osborne, 1993). In many mammalian cells the effectors for apoptosis are continuously present and are activated by several mechanisms (Weil et al., 1996).

Apoptosis can clearly be distinguished from necrosis, the latter involving lysis of cells and organelles and collateral tissue responses. By contrast, characteristic features of apoptosis are the death of individual cells with condensation of chromatin and cytoplasm with relatively well-preserved organelles, followed by fragmentation of the cell and phagocytosis of the apoptotic bodies by macrophages or neighboring cells (Steller, 1995; Wyllie et al., 1980) (Fig. 29-3). Besides light- and electron microscopy, there is the histochemical demonstration of apoptosis by in situ labeling of DNA nick ends by the TUNEL method (terminal transferase-dUTP nick-end labeling) (Gavrieli et al., 1992) or modifications thereof. During apoptosis, the nuclear DNA is fragmented into regular subunits of about 200 base pairs (bp) or their multiples and these fragments can be visualized by gel electrophoresis forming the so-called ladder. For unequivocal confirmation of apoptosis, at least two of those methods should demonstrate the described changes.

The expression of several genes coincides with apoptosis, for example, stromelysin, ubiquitin, clusterin, and others. The expression of other genes appears to be involved in the regulation of cell death, either preventing or promoting the death program, for example, c-myc, glucocorticoid receptor, p53, bcl-2, and others (Gavrieli et al., 1992; Reed, 1994; Schwartz et al., 1993; Steller, 1995). There is a wide variety of tissues in which apoptosis occurs and an equally large number of endogenous and exogenous stimuli and mediators (Schwartzman and Cidlowski, 1993; Thompson, 1995). Intracellular signaling pathways may include increased calcium levels, protein kinase C (PKC), phosphatidylinositol-3-kinase (PI-3 kinase), oxidative stress and lipid hydroperoxides, and activation of endogenous endonucleases. Recent evidence indicates that the small family of transcription factor proteins Fos and Jun (AP-1) may regulate apoptosis in several systems (Colotta et al., 1992; Hafezi et al., 1997b; Marti et al., 1994; Preston et al., 1996). An important regulative role for apoptosis is also ascribed to the transcription factor proteins Myc and Max (Amati et al., 1993). To date, numerous effector molecules have been identified that are essential for the basic death program. Intracellular proteases and nucleases are thought to be crucial components. For example, family members of the protease IL-1β-converting enzyme (ICE) appear to be key enzymes to execute apoptosis (Martin et al., 1995).

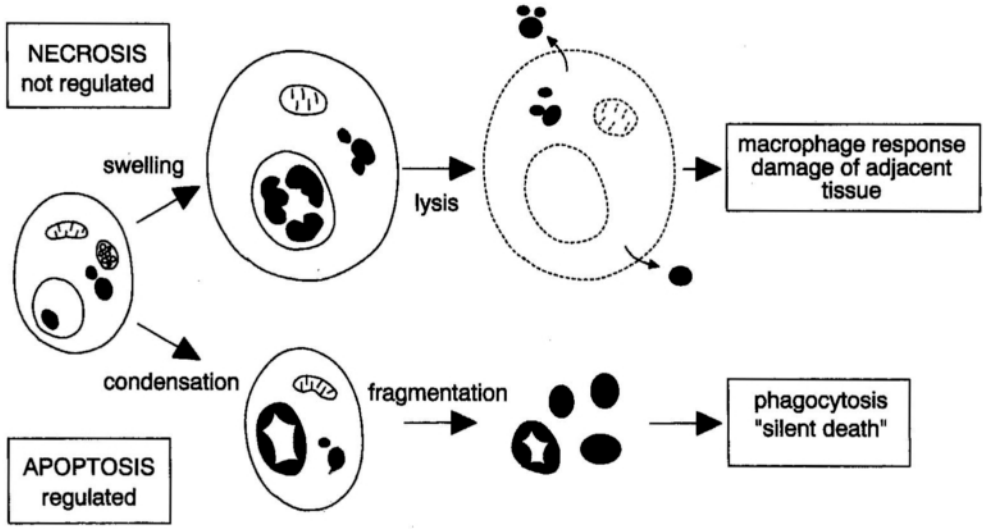

FIGURE 29-3. Schematic drawing illustrating the different modes of cell death in necrosis versus apoptosis. Necrosis includes swelling and disruption of cells and organelles, causing collateral reactions in the adjacent tissue. Apoptosis, by contrast, implies condensation and shrinkage of nucleus and cytoplasm followed by fragmentation and phagocytosis of fragments by adjacent cells or macrophages.

Retinal Dystrophies

In the eye, apoptosis is observed in the developing mouse retina (Young, 1984) and in rat retinas that have been exposed to lead during development (Poblenz et al., 1995), in retinoblastoma (Howes et al., 1994), and in some animal models of retinitis pigmentosa (Chang et al., 1993; Gregory et al., 1995; Lolley, 1994; Papermaster et al., 1995; Portera-Cailliau et al., 1994; Tso et al., 1994; Wong et al., 1995b). Furthermore, apoptosis is found in donor eyes from patients that had suffered from retinitis pigmentosa (Li and Milam, 1995). The discovery of apoptosis in those animal models with diverse genotypic and phenotypic characteristics may indicate a final common pathway during the course of the retinal dystrophy. Which gene mutations and their molecular consequences acutally may induce the apoptotic death pathway is unknown. Several genes have been investigated for a potential involvement in retinal apoptosis, such as clusterin, *c-fos,* and p53 (Wong et al., 1995a; review: Remé et al., 1998).

Several factors induce apoptosis in the pigment epithelium in vitro, such as tumor necrosis factor-α (TNF-α, staurosporine, anti-Fas antibody). Apoptosis was prevented by bFGF and PDGF (He et al., 1995). The well-know photosensitizer and antidepressant hypericin, contained in St. John's wort, or *Hypericum perforatum* (Duran and Song, 1986), induces apoptosis in the PE at higher concentration, possibly via an inhibition of PKC.

Light-Induced Apoptosis in Retina and Pigment Epithelium

It is noteworthy but perhaps not surprising that apoptotic cell death in the retina can be evoked by light. Exposure to intense green light for prolonged periods (Abler et al., 1996) and exposure to diffuse white light of relatively low intensity for short time periods (Remé et al., 1995b) elicits an apoptotic response. The amount of apoptosis in the retina is strain dependent (Lai et al., 1995), can be ameliorated by different agents (Chang et al., 1994), and occurs sooner after exposure to intermittent light than continuous light of the same final irradiant dose (Li et al., 1994).

In our model of acute threshold light damage in the albino rat, apoptosis in photoreceptors and pigment epithelium is seen in the lower central retina after exposure of dark-adapted animals to diffuse white fluorescent light of 1000 lux for two hours, whereas even more apoptosis occurs after exposure to 3000 lux for two hours. In the retina of pigmented mice, distinct apoptosis is seen after exposure to 5000–6000 lux for two hours (Fig. 29-4a, b). Addressing the question of apoptosis in cones, we observed ample apoptotic cells in the retina of the thirteen-lined ground squirrel, which has an all-cone retina, in animals living in a normal light/ dark cycle shortly before entering hibernation (Fig. 29-3c).

Detailed time course studies show that in the rat retina a massive and immediate apoptotic response at the end of light exposure is followed by an almost complete

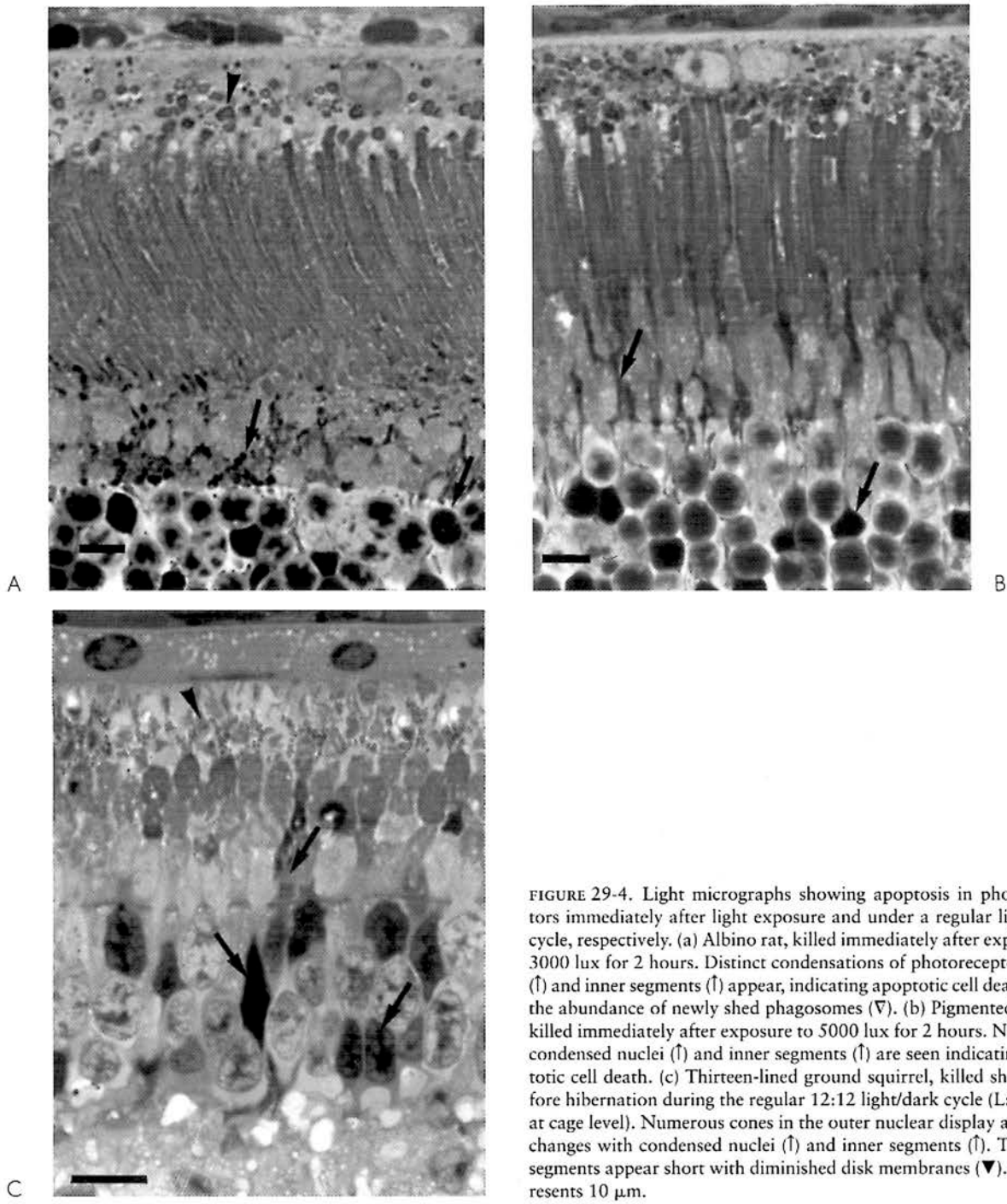

FIGURE 29-4. Light micrographs showing apoptosis in photoreceptors immediately after light exposure and under a regular light/dark cycle, respectively. (a) Albino rat, killed immediately after exposure to 3000 lux for 2 hours. Distinct condensations of photoreceptor nuclei (↑) and inner segments (↑) appear, indicating apoptotic cell death. Note the abundance of newly shed phagosomes (∇). (b) Pigmented mouse, killed immediately after exposure to 5000 lux for 2 hours. Numerous condensed nuclei (↑) and inner segments (↑) are seen indicating apoptotic cell death. (c) Thirteen-lined ground squirrel, killed shortly before hibernation during the regular 12:12 light/dark cycle (L: 100 lux at cage level). Numerous cones in the outer nuclear display apoptotic changes with condensed nuclei (↑) and inner segments (↑). The outer segments appear short with diminished disk membranes (▼). Bar represents 10 μm.

decay of photoreceptors about 24–36 hours after light exposure. This is confirmed by gel electrophoresis. TUNEL labeling reveals distinct staining within the entire outer nuclear layer, in contrast to the staining of fewer cells at earlier time points (Fig. 29-5a–c). At early time points during and after light exposure, such as 30, 60, 90 minutes *during* exposure and 0, 60, 120 minutes *following* exposure, electron micrographs show clear signs of chromatin condensation and inner segment densifications in photoreceptors, but gel electrophoresis is negative for apoptotic signs (Fig. 29-6a–i). TUNEL staining reveals few positive nuclei in the outer nuclear layer at zero hours after exposure, distinctly fewer positive cells than morphology reveals. This dis-

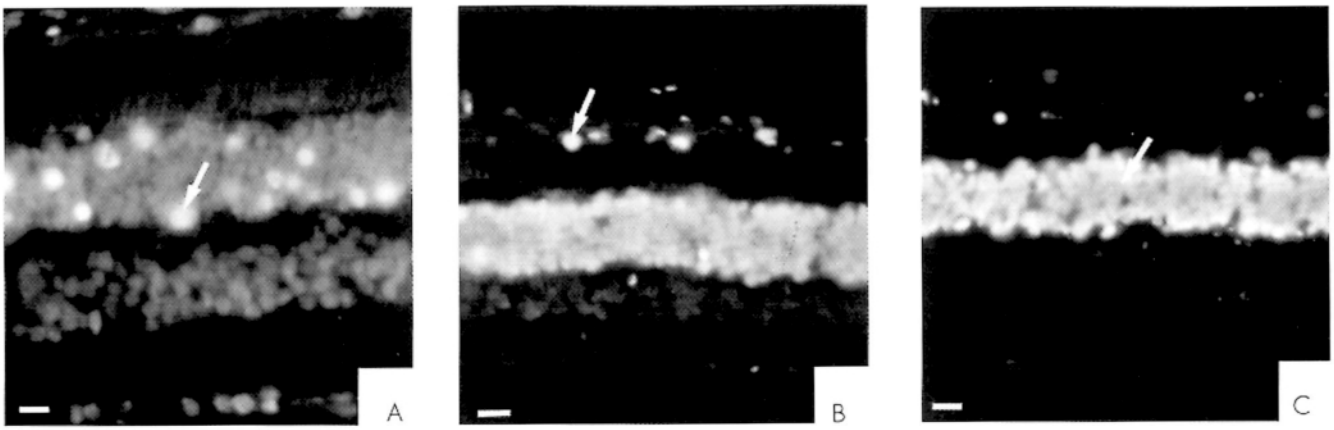

FIGURE 29-5. TUNEL staining demonstrating apoptosis at different times after exposure to 3000 lux for 2 hours in the albino rat retina. (A) Positive TUNEL staining of some nuclei (↑) in the ONL 5 hours after exposure. Bar represents 10 μm. (B) Positive TUNEL staining of nuclei (↑) in the PE 10 hours after exposure. Bar represents 20 μm. (C) Massive TUNEL staining of the ONL 24 hours after exposure. Bar represents 20 μm.

crepancy may indicate that DNA fragmentation occurs in several steps with a cleavage into large fragments in the kilobase range preceding the internucleosomal fragmentation that produces the positive nick-end labeling and the ladder in gel electrophoresis. This has been observed by other laboratories in nonocular tissues (Cohen et al., 1992; Oberhammer et al., 1993) and in the lead-exposed developing retina (Poblenz et al., 1995). The endonucleases performing DNA fragmentation have not yet been characterized in detail, DNase-I may be involved (Peitsch et al., 1993).

The morphological picture of massive and rather simultaneous decay of photoreceptors following at 24 hours after bright light exposure may be interpreted as a tissue necrosis, because macrophages are beginning to invade the area of lesion and only a few viable cells can be distinguished. However, recent studies in our laboratory using mice lacking the gene *c-fos* clearly demonstrate a complete prevention of acute and delayed apoptosis in the retina after exposure to 5000-lux for 2 hours. Therefore, the massive cellular decay seen in the rat model and in control wild-type mice is interpreted as apoptotic (Hafezi et al., 1997b).

In notable contrast to photoreceptors, the pigment epithelium shows a different time course of apoptosis. In electron micrographs, peripheral chromatin clumping followed by cytoplasmic condensation is seen at 5–24 hours after light exposure. TUNEL staining shows distinctly positive PE nuclei about 10 hours after exposure. These different timing patterns in the retina and the PE may indicate that trigger mechanisms, messengers, and gene expression are not identical (Fig. 29-7). Possibly, the rapid death of photoreceptor cells represents a major mechanism to trigger apoptosis and cell death in the PE. Furthermore, following light exposure, cytokines may be induced in the retina and/or the PE, which could trigger a delayed apoptotic response (see "Fatty Acid Metabolites . . ." above) (Hafezi et al., 1997a). In nonocular systems, cytokines act as proapoptotic factors (Han et al., 1996).

Potential Gene Expression, Messengers, and Inducers in Light-Evoked Apoptosis

As mentioned earlier, there is a multitude of agents that can induce apoptosis in nonocular tissues, whereas for the eye, these processes are just beginning to be investigated. Similarly, potential messengers and potential genes involved in apoptosis are barely unraveled. Light damage studies in the zebrafish by means of microscopy, western blotting, and differential display polymerase chain reaction (DDPCR) disclosed novel genes potentially involved in light damage. Furthermore, the transcription factors c-Fos and c-Jun were elevated (Robinson et al., 1995). In the rat retina, the expression of clusterin correlated with light damage and the levels of expression reflected the severity of lesions (Wong et al., 1995a). In the normal mouse retina, *c-fos* is expressed in a diurnal manner with high levels in the dark but not in the light (Nir and Agarwal, 1993; Yoshida et al., 1993), whereas in the *rds* mouse, the levels of c-Fos were high throughout the light/dark cycle, indicating that it may be a signal for the apoptotic pathway (Agarwal et al., 1995). A detailed study of the expression of c-Fos was performed in the *rd* mouse. An aberrant expression of the c-Fos protein was found in photoreceptors im-

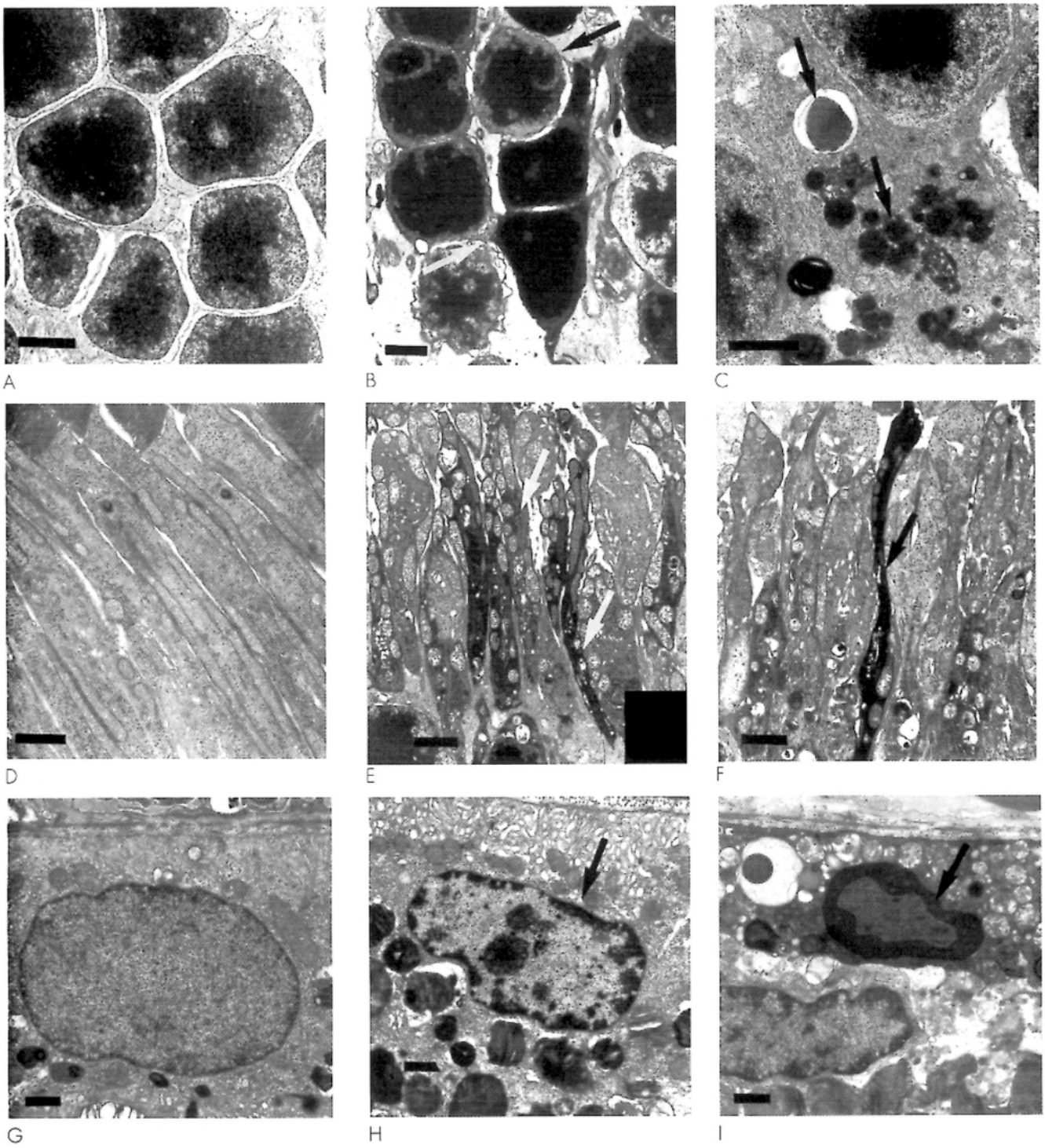

FIGURE 29-6. Electron micrographs depicting apoptotic changes in photoreceptor and PE nuclei and cytoplasm at different time points after exposure to 3000 lux for 2 hours in the albino rat retina. (a) Unexposed control nuclei of photoreceptors showing the regular pattern of chromatin. Bar represents 2 μm. (b) Early (↑) and intermediate (↑) ...nents (↑). Bar represents 2 μm. (f) Late stage ...es (↑) among normal photoreceptor nuclei. Bar represents 2 μm. Unexposed control photoreceptor inner segments. Bar represents 1 μm. (e) Early stages of cytoplasmic condensation of photoreceptor inner segments (↑). Bar represents 2 μm. (f) Late stage of cytoplasmic condensation of a photoreceptor inner segment (↑). Bar represents 2 μm. (g) Unexposed control nucleus of the PE. Bar represents 3 μm. (h) Early stage of peripheral chromatin condensation (↑) ...clear chromatin condensation (↑) and cytoplasmic condensation in a PE cell. Bar represents 2 μm. (c) Apoptotic bod... resents 2 μm. (d)

photoreceptor and PE nuclei and cytoplasm at different time points

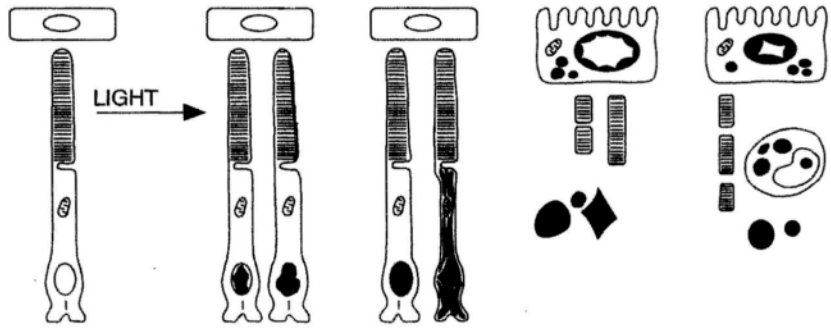

FIGURE 29-7. Schematic drawing summarizing an approximate time course of apoptotic changes in photoreceptors and PE after exposure to 3000 lux for 2 hours in the albino rat retina. Morphological signs of ONL chromatin condensation occur as early as 30 minutes after the onset of light exposure followed by cytoplasmic condensation at 60 minutes during light exposure. 5 hours after exposure apoptotic bodies appear in the ONL and progressive chromatin condensation occurs in the PE. 24 hours and later, the PE shows distinct apoptotic signs; the ONL reveals massive cellular decay, apoptotic bodies, and macrophages. TUNEL staining appears in the ONL from 5 hours after exposure and from 10 hours after exposure in the PE. Ladder formation in gel electrophoresis is seen from 5 hours after exposure.

mediately prior to their death by apoptosis, suggesting the possibility that c-Fos may be involved in triggering apoptosis (Rich et al., 1997). In several tissues, the gene *bcl-2* was found to protect against apoptotic cell death (Reed, 1994), possibly among other mechanisms via antioxidant pathways (Hockenbery et al., 1993). In mice with inherited retinal degenerations, *bcl-2* overexpression slightly retarded the apoptotic death of photoreceptor cells. Similarly, *bcl-2* overexpression diminished but did not prevent photoreceptor death in mice exposed to constant light (Chen et al., 1996).

Studies in our laboratory using mice lacking a functional protooncogene *c-fos* (c-fos knockout mice) revealed a striking scarcity of apoptosis in knockout mice after 2 hours of exposure to 5000 lux white fluorescent light and the complete absence of severe lesions in retina and PE at 12 and 24 hours after light exposure. By contrast, control littermates showed distinct and irreversible lesions (Hafezi et al., 1997b). Those studies imply a functional role of *c-fos* in retinal apoptosis and show a complete protection against light-induced cell loss in the absence of *c-fos*. In view of those studies, apoptosis may be considered as a major underlying mechanism of retinal light damage. To date, the exact role of *c-fos* in the apoptosis pathway is not known. It is conceivable that *c-fos* or the lack of *c-fos* modify retinal physiology and thus alter the susceptibility to light-induced apoptosis. Alternatively, *c-fos* may be a specific gene for some apoptosis pathways.

The cysteine protease IL-1β-converting enzyme (ICE) is thought to play a key role in apoptosis in nonocular tissues and corresponds to the cell death gene *ced-3* of the nematode, *Caenorhabditis elegans*. ICE may activate other proteases and/or endonucleases but may also have further unidentified functions in the pathway of apoptosis (Allen et al., 1995; Vaux et al., 1994). Thus, it will be important to investigate whether the ICE family of proteases is involved in retinal apoptosis.

In nonocular tissues, oxidative stress was found to mediate apoptotic cell death (Buttke et al., 1994; Ratan et al., 1994; McGowan et al., 1996). Furthermore, enzymatically peroxidized metabolites of arachidonic acid were found to induce apoptosis in various experimental models (Agarwal et al., 1993; Haliday et al., 1991; Horiguchi et al., 1989; Sandstrom et al., 1994). Because oxidative stress is thought to be one of the hallmarks in the course of retinal and PE light damage, this process is a likely candidate for the induction of apoptosis in light-induced lesions. Notably, light can release arachidonic acid and docosahexaenoic acid and its metabolites in the retina (Jung et al., 1994; Reinboth et al., 1996; Reinboth et al., 1995). Indeed, preliminary studies in our laboratory revealed an induction of apoptosis by AA metabolites in the retina in vitro (Hafezi et al., 1998). Thus, reactive oxygen species as well as PUFAs and their metabolites may well subserve important messenger functions in the apoptosis pathway in retinal light damage (Fig. 29-2). The suppression of light-induced apoptosis by antioxidants, by oxidative enzyme inhibitors, or by phospholipase inhibitors will strength-

en this notion. The light-evoked retinal messengers may also act as messengers for the PE. This can be tested in experimental models using PE cells in culture.

LIGHT DAMAGE IN THE CONTEXT OF HUMAN RETINAL PATHOPHYSIOLOGY

Are there relevant relations between light damage and human retinal pathophysiology? One must distinguish acute and chronic light-induced lesions in order to answer this question. The solar retinopathy is a typical example of an acute lesion that may or may not leave a small central scar and functional disturbances. The pathogenesis of cystic macular edema and proliferative vitreoretinopathy (PVR) is a subject for intense discussion. In view of our observations that light can release cellular mediators in the retina and light exposure creates an apoptotic response with macrophage invasion in the retina and PE potentially followed by the release of cytokines, a causal relation seems plausible but needs further experimental and clinical support.

An intriguing but unanswered question is whether exposure to light during a lifetime can accelerate or even partially cause aging changes in the retina and PE. Detailed studies in autopsy eyes demonstrate a selective loss of rods in the central fundus while the number of cones remains remarkably stable. It is tempting to speculate that light exposure is a causative or contributory factor for this phenomenon. The question then arises, however, why rods but not cones are diminished with age even though they are likely to receive the same amount of light and are in a similar metabolic situation (Curcio et al., 1993). An analogy may be found in the rat retina, where cones survived significantly longer from the exposure to constant bright light (LaVail, 1976). Rods may die through an apoptotic mechanism triggered among other potential causes by light and the threshold for apoptosis may be lower in rods than in cones. In aging Fisher rats, cell loss includes apoptosis (Fan et al., 1995). However, a relation of age-related rod loss with light exposure remains to be elucidated.

The accumulation of PE lipofuscin, the retinal "age pigment," has been tentatively related to light exposure (Weale, 1989). The steep increase in PE lipofuscin content during the first two decades of life may be due to a high lens transmission, with increased exposure of the retina to short wavelength visible light and UVA. The second rise in lipofuscin content from the fifth decade onward that follows a plateau may be the result of augmented light exposure due to an increase in lens transmission by means of a dislocation of the mainly absorbing nucleus (Weale, 1989).

Bruch's membrane shows distinct alterations with age and some of them include inflammatory signs such as macrophages (Bird, 1992). Again it is open for speculation whether light-evoked inflammatory mediators and cytokines contribute to such changes.

What is the role of light exposure in retinal degenerations and dystrophies? There is much discussion about the potential role of light in the pathogenesis of age-related macular degeneration (AMD) (Young, 1987; 1988). It is well known that AMD is a multifactorial disease that may be associated with environmental and endogenous risk factors. However, genetic disposition may also contribute. Indeed, mutations of the Stargardt disease gene have now been observed in 16% of a population of unrelated AMD patients (Allikmets et al., 1997). Despite of a lack of direct epidemiological or clinical evidence, laboratory studies suggest the possibility that light may be a contributory environmental factor.

Geographic Atrophy

The "silent death" by apoptosis may diminish photoreceptors and PE, leaving the well-recognized central geographic atrophy in AMD. In the macula, apoptosis would have to include rods and primarily cones. Pilot studies in our laboratory in the thirteen-lined ground squirrel clearly show apoptotic cell death of cones, indicating that cones can indeed die by apoptotic mechanisms (Fig. 29-3c). The signalling for apoptosis remains to be investigated, but light exposure initiating a messenger cascade cannot be excluded (Fig. 29-2).

Deposits and Drusen

Deposit and drusen formation in the area of Bruch's membrane may be considered from a novel vantage point in the context of apoptosis and AMD. In nonocular epithelial and endothelial cells, apoptosis is induced by deprivation of extracellular matrix anchorage and extracellular signaling molecules (Frisch and Francis, 1994; Meredith et al., 1993; Ruoslahti and Reed, 1994). With drusen and deposits in Bruch's membrane, a similar deprivation mechanism could act on PE cells, causing them to undergo apoptotic death with subsequent loss of overlying photoreceptors. This notion is supported by observations that demonstrate apoptotic cell death in photoreceptors of mice deficient for the adhesion molecule on glia (AMOG) (Molthagen et al., 1996). Detailed mechanisms of formation of drusen and deposit are unknown but PE-cell-derived cytoplasmic debris may represent a pathogenetic factor: The accumulation of lipofuscin granules and other phagocytic

material in the PE has long been suspected to contribute to the formation of drusen. In view of lipofuscin as a candidate photosensitizer in light damage which initiates free-radical reactions upon light exposure, such a cause for cellular dysfunction or death seems plausible. This cell death may include apoptosis. The described mechanisms would imply a self-sustaining vicious circle between drusen and deposit formation on the one hand and apoptosis on the other hand.

Subretinal Neovascularization

Chronic inflammatory and proliferative changes may be induced and maintained via the release of inflammatory lipid mediators, mononuclear cells, and cytokines, and the cascade may be initiated by light exposure. Subretinal neovascularization may be stimulated when inflammatory cytokines and VEGF predominate. Several conditions are known to evoke VEGF (see "Fatty Acid Metabolities . . ." above). Retinal neovascularization was inhibited when the adhesion receptor system for newly forming vessels such as various integrins was inhibited (Hammes et al., 1996).

The route and end stage of AMD—atrophic or proliferative—may depend on genetic factors, ocular defense systems, and exogenous factors such as light exposure and others.

Animal Models of Human Retinitis Pigmentosa

Apoptosis was recently discovered and assumed to represent a final common death pathway in diverse genetic mutations in animal models for human retinitis pigmentosa. Because light can induce apoptosis in the human retina and PE and light exposure was shown to induce apoptosis genes in animals (Hafezi et al., 1997b), light exposure of humans or animal models suffering from retinitis pigmentosa may thus lead to an acceleration of apoptotic cell death. This assumption can be tested in the laboratory and may have distinct clinical significance for retinitis pigmentosa patients.

CONCLUSION

An important and perhaps underestimated insight thus emerges from recent research on retinal light damage and light-induced apoptosis: the retina and the PE possess the inherent property to respond to light exposure with the release of cellular mediators, signaling molecules, and cell death by apoptosis. When several of those factors act in concert, such as light exposure and surgical trauma, light exposure and an insufficient defense system, light exposure and photosensitization, or bright light exposure to a completely dark-adapted eye, pathological cascades may be initiated and may lead to retinal dysfunction. On these bases, new therapeutic strategies can be developed from the knowledge of cellular mediators and regulative mechanisms of apoptotic cell death.

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