Basic Sciences

Principles and Practice Of Ophthalmology

Eds. D M ALBERT, F A JAKOBIEC

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Note error in caption to Figure 116-13. The Noell damage should be solid squares; the Ham, damage solid circles; the rat damage, solid triangles and the bovine RPE potential, solid stars.

Chapter 116

Light Effects on the Retina

JOHN MELLERIO

The retina has evolved especially to be sensitive to light. The way it responds to light as part of the visual process is not the concern here-that is for the physiologist and for earlier chapters. The manner in which the retina interacts with light to cause deleterious changes in function is the substance of this chapter.

Two problems often stand out for those who work on light-induced retinal damage. These are the proper quantification of the light insult and the detection of changed function. Specifying and understanding the exact nature of the stimulus to damage should not be a problem if the physics is considered. The following discussions attempt to ease the reader through the complexities of the two different systems of units that are used, sometimes incorrectly, to specify the stimulus. The way in which functional deficits are detected are not considered here; this can be learned from texts concerned directly with vision. Often, techniques that search for morphologic changes are substituted for functional tests, as the lesions are small, and the resolution of behavior studies cannot pick up the small lesions that may be produced in the retinas of animals that are not renowned for their ability to perform in laboratory studies. Histologic techniques, like those of testing vision, also are not discussed. Rather, consideration is given to stimuli and the effects they produce.

QUANTIFYING THE SOURCE

The electromagnetic (EM) energy that is our subject is termed radiation, and its importance lies in its capacity to bring about effects in the world. It has parameters of intensity and wavelength (or frequency), which, together with certain properties of the substrate. determine what effects it can produce.

At intensities that might be described as everyday and at which nonlinear effects do not occur, some fraction of the incident radiation must be absorbed in the substrate before any effect can occur. The extent of the effect depends on the intensity of the radiation and on the substrate's absorption and sensitivity. Consequently, the task of predicting the size of an effect might start by considering the quality and intensity of the radiation across the part of the spectrum of interest. These depend on the source of the radiation.

The quality of the radiation includes the parameters of wavelength, coherence, geometric distribution, and temporal characteristics (i.e., whether continuous or pulsed). Table 116-1 shows the way in which the EM spectrum is conventionally divided into regions that delimit the spread of wavelengths having similarities in the way they behave with substrates. Strictly, only visible radiation may be called light, but often the term is loosely applied to include ultraviolet (UV) and infrared (IR) radiation. The energy output of a source varies with wavelength and is shown in a spectral power distribution (SPD) plot (i.e., power versus wavelength). Solid incandescent sources have SPDs that usually approximate that of a black body or planckian radiator, which means that peak emission occurs at a wavelength that decreases with increasing temperature. Indeed, the radiation characteristics can be described by the three radiation laws of Planck, Stefan-Boltzmann, and Wien. It is the last

that relates peak wavelength to temperature:

$$l_{peak} = \frac{b}{T} \qquad (1)$$

where b is Wien's constant (2897 μm K) and T is the temperature (in kelvins) of the source. Often, light sources are specified in terms of color temperature: for ideal black bodies, they are the same as the physical temperature, but for bodies with an emissivity that varies with wavelength they are not the same. At temperatures that may be realized in practical filament lamps-for example, 3360 K for a photoflood - λ_{peak} is about 860 rim and the particularly hazardous blue and UV radiation output is insignificant.

In coherent radiation the photons are of equal wavelength, phase, and polarization: Lasers emit this kind of radiation, but only the monochromaticity is relevant to this discussion. The high intensity of laser sources stems largely from their efficiency and their geometry, which usually confines their output to a narrow beam of energy. When compared with conventional incandescent or discharge sources, lasers are particularly useful to instrument designers, because their geometry is favorable for coupling their radiation to optical systems. Continuous wavelength (CW) or pulsed sources deliver energy with temporal characteristics that cannot be ignored and that are described later.

The “intensity” or energy content of a source is quantified in units that belong to one or the other of two systems. The first and more fundamental is the *radiometric system*, which is concerned only with the energy content of the radiation; these units are physically absolute in the sense that they do not depend on the spectral response of a detector. The second is the *photometric system*, which relates the energy to the spectral sensitivity of the eye. The photometric system of units has, in effect, built in the action spectrum for vision. The action spectrum of most universal interest is that defined by the photopic visibility curve, which is termed the V_λ function and relates to vision by the cone system of the retina. Consequently, there are photopic units of radiation that measure visible radiation, which is, by definition, light. There exists a parallel system of light units based on the action spectrum for scotopic vision, which is mediated via the rods. Scotopic units are usually confined to vision research laboratories or to consideration of retinal damage mechanisms in which rhodopsin is thought to be the primary causative agent. The scotopic visibility or V'_λ function is the action spectrum that applies.

It is obviously important that the correct system of units be used and, within each, the appropriate unit. Table 116-2 summarizes the radiometric and photometric units that are commonly used and clearly shows the parallel nature of many of the units in the two systems. The *brightness* of a source is described by the terms "radiance" and "luminance," respectively, in the radiometric and photometric systems. These terms specify the rate at which radiant or light energy leaves the source in a given solid angle from a unit surface area of the source, and the units are as set out below:

Table 116–1. THE DIVISION OF THE SPECTRUM OF OPTICAL RADIATION INTO DOMAINS WITH SIMILAR PATHOPHYSIOLOGIC ACTION

Spectral Domain	Wavelength (nm)	Tissue	Absorption Site	Nature of Damage
Ultraviolet C	200–280	Cornea	Epithelium	Photochemical: photokeratitis, corneal opacity
Ultraviolet B	280–315	Cornea	Epithelium	Photochemical: photokeratitis, corneal opacity
	295–315	Lens	Nucleus	Photochemical: cataract
Ultraviolet A	315–400	Lens	Nucleus	Photochemical: cataract(?)
Visible	400–780	Retina	RPE	Thermal (thermoacoustic): vision loss, intraocular hemorrhage
			Hemoglobin	Thermal: vision loss, intraocular hemorrhage
			Macular pigment	Thermal: central vision loss
			RPE, visual cells	Photochemical: insidious vision loss, color vision problems
Infrared A	780–1400	Retina	RPE	Thermal: vision loss
		Lens	Epithelium	Thermal: cataract
Infrared B	1400–3000	Cornea	Epithelium, stroma	Thermal: corneal opacity
		Lens(?)	Epithelium	Thermal: cataract
Infrared C	3000–10,000	Cornea	Epithelium	Thermal: superficial burns

RADIANCE

Joule per second per steradian per square meter, which simplifies to Watt.sr^{-1}.m^{-1}. The defining equation is

$$L_e = \frac{d^2\Phi_e}{d\Omega \cdot dA \cdot \cos q} \qquad (2)$$

where Φ_e is the radiant flux, Ω the solid angle, and A the surface area of the source. It also includes the cosine law, which allows for measurement at an angle θ from the normal to the surface of the source because the projected unit area along the line of measurement decreases with the cosine of θ.

LUMINANCE

Lumen per steradian per square meter, which becomes candela per square meter (cd.m^{-1}), as a candela is a lumen per steradian. The defining equation is

$$L_v = \frac{d^2\Phi_v}{d\Omega \cdot dA \cdot \cos q} \qquad (3)$$

and is the same as for the radiance except that the flux term in the numerator is the luminous flux, which contains the all-important luminous efficiency of radiation, K_m in its definition:

$$\Phi_v = K_m \int \frac{d\Phi_e}{dl} V_l \, dl \qquad (4)$$

The value of K_m is usually taken as 680 lumens per watt and is defined as the maximum number of lumens produced by 1 watt of broadband radiation extending from the UV to the IR (i.e., over the visible range of the spectrum) in the relation:

Table 116–2. COMMON RADIOMETRIC AND PHOTOMETRIC TERMS AND UNITS

Term	Symbol	Lay Definition	Defining equation	SI Units
Radiometric				
Radiant energy	Q_e	Energy emitted		Joule (J)
Radiant energy density	W_e		$W_e = \frac{dQ_e}{dV}$	Joule per cubic meter (J.m^{-3})
Radiant power (radiant flux)	ϕ_e		$\phi_e = \frac{dQ_e}{dt}$	Watt (W)
Irradiance (radiant flux density)	E_e	Rate at which energy falls on or passes through an area, i.e., dose rate	$E_e = \frac{d\phi_e}{dA}$	Watt per square meter (W.m^{-2})
Radiant intensity	I_e		$I_e = \frac{d\phi_e}{d\Omega}$	Watt per steradian (W.sr^{-1})
Radiance	L_e		$L_e = \frac{d^2\phi_e}{d\Omega \,.\, dA \,.\, \cos\theta}$	Watt per steradian per square meter (W.sr^{-1}.m^{-2})
Radiant exposure	H_e	Term for dose in photobiology	$H_e = \frac{dQ_e}{dA}$	Joule per square metre (J.m^{-2})
Optical density	D_e	A measure of attenuation of light	$D_e = -\log_{10}\tau_e$	Unitless
Photometric				
Quantity of light	Q_v	Lumen second (lm.s)	$Q_v = \int \phi_v \,.\, dt$	
Luminous energy density	W_v	Lumen second per cubic meter (lm.s.m^{-3})	$W_v = \frac{dQ_v}{dV}$	
Luminous flux	ϕ_v	Lumen (lm)	$\phi_v = 680 \int \frac{d\phi_e}{d\lambda} V(\lambda)\, d\lambda$	
Illuminance	E_v	Lumen per square meter (lm.m^{-2}) or lux	$E_v = \frac{d\phi_v}{dA}$	
Luminous intensity (candle power)	I_v	Lumen per steradian (lm.sr^{-1}) or candela (cd)	$I_v = \frac{d\phi_v}{d\Omega}$	
Luminance	L_v	Candela per square meter (cd.m^{-2})	$L_v = \frac{d^2\phi_v}{d\Omega \,.\, dA \,.\, \cos\theta}$	
Light exposure	H_v	Lux-second (lx.s)	$H_v = \frac{dQ_v}{dA} = \int E_v \, dt$	
Optical density	D_v	Unitless	$D_v = -\log_{10}\tau_v$	

$$K_m = \frac{\Phi_v}{\Phi_e} \qquad (5)$$

The maximum value is taken for K_m as the ratio in equation 5 varies with the spectral distribution of energy (e.g., radiation with a relatively large amount of IR energy is not visually very effective). K_m is the link between radiometric and photometric units.

The *rate* at which energy arrives at a substrate has corresponding units in each system.

IRRADIANCE

Energy per time per unit area, which is usually measured in watts per square meter ($W.m^{-2}$) , as a watt is a joule (J) per second.

ILLUMINANCE

Also energy per time per unit area, but the units are lumens per square meter ($lm.m^{-2}$) , as lumens are defined in terms of Φ_e which itself is a time flux of energy.

An important radiometric term is that of radiant energy density ($J.m^{-1}$), which defines the amount of energy within a certain volume of substrate that is available to influence the substrate. Often the term "energy density" is used incorrectly when the radiant exposure ($J.m^{-2}$) is meant. This latter unit is easier to measure or calculate but is not always as useful as many of those who employ it think. The corresponding photometric unit of luminous energy density ($1m.s.m^{-3}$) is not often used.

For completeness the troland (td) must be added to the units in the table. This is a unit of retinal illumination defined as the product of the source luminance ($cd.m^{-1}$) and the pupillary diameter squared (mm^2). The troland is used in studies of retinal photochemistry and various aspects of visual performance.

Figure 116-1 summarizes some of the concepts that underlie the radiometric units described above.

QUANTIFYING THE DOSE

The dose, the quantity of energy deposited in a substrate, depends on three parameters: the "intensity" of the radiation, the substrate's absorbing properties, and the duration of exposure.

Intensity and absorption are dealt with by the process of "convolution." The absorption of the substrate is usually determined at each wavelength, in the region of the EM spectrum of interest, to give the

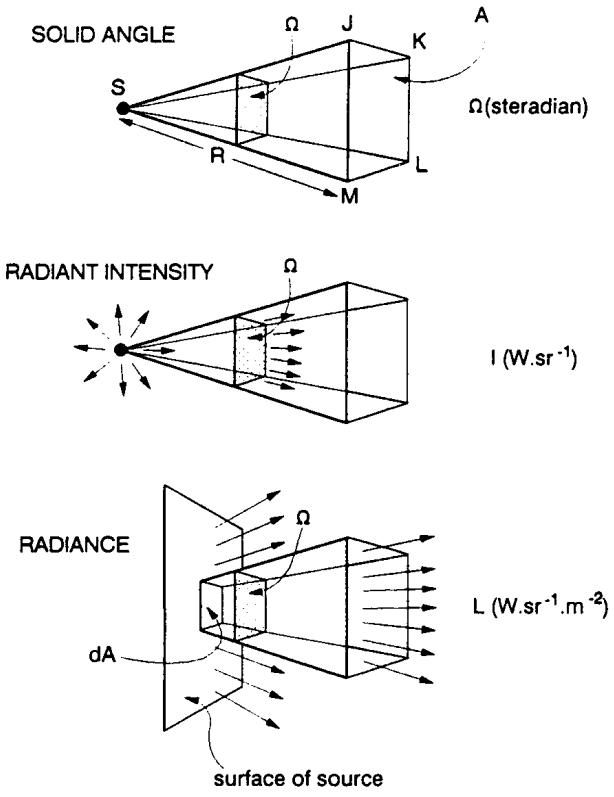

Figure 116-1. Summary diagrams of some concepts of radiometry: The photometric system has similar concepts. The solid angle is shown bounded by a square; the cross section may have any shape and is shown here as square for clarity. *Solid angle:* The area A bounded by *JKLM* subtends a solid angle Ω steradians at the source S defined as $\Omega = A/R^2$ steradians. *Radiant intensity:* The source emits a power of Φ in all directions and delivers into the solid angle Ω a reduced quantity known as the radiant intensity defined as $I = d\Phi/d\Omega$ with units of watts per steradian. *Radiance:* Each small area dA of the surface of an emitting source emits power into a solid angle of Ω: this is the radiance defined as $L = d^2\Phi/dA.d\Omega$ with units of watts per steradian per m^2.

spectral absorption curve. This curve is multiplied, wavelength by wavelength, by the spectral power distribution of the radiation source. This convolution process yields the *relative absorbed spectral dose curve*, which specifies the energy deposited in the substrate across the spectrum. If the intensity of the radiation source is increased, the area under this curve increases also. Indeed, this area, is the spectral integral defined so:

$$Q_{max} = \sum_{l_1}^{l_2} a_l \cdot p_l \, (dl) \qquad (6)$$

where a_λ and p_λ are the spectral absorption and spectral power and represent the maximum possible energy that is available to influence the substrate. It is a useful, but necessarily rough, guide in comparing the effects of different radiation sources on one substrate, or how different substrates might behave if exposed to the same radiation source.

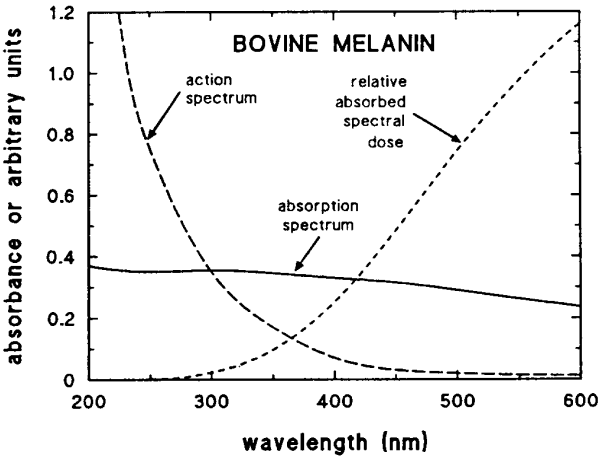

Figure 116-2. The **absorption spectrum** for bovine melanin in solution in KOH (solid line) is relatively flat and has given rise to the idea that melanin is a broad-band absorber. The relative absorbed spectral dose is the absorption value multiplied by the power output of a 1000-W tungsten halogen quartz iodide lamp for each wavelength and shows that the energy trapped in the melanin is mostly of the longer wavelengths. The action spectrum shows how much energy is required at each wavelength to produce a given uptake of oxygen by melanin in saline suspension. (Biologic data from Sarna and Sealy[14]; lamp data from Sliney and Wolbarsht.[70])

The relative absorbed spectral dose curve, then, is specific for substrate and radiation source but is not necessarily the same as the action spectrum, which is an experimentally determined, criterion-referenced function. In an action spectrum a certain magnitude of response is chosen and then the energy required at each wavelength to produce this response is recorded. The action spectrum is perhaps the most useful specification of spectral behavior, as it is tied to an effect (but see reference 1), whereas the relative absorbed spectral dose curve may include energy that is absorbed by the substrate but has no part in any processes other than wasteful degradation to heat. Figure 116-2 shows the absorption curve, the relative absorbed spectral dose curve, and the action spectrum for bovine melanin: It is immediately obvious that there are considerable differences among the three. The reasons for the differences lie in the very nature of the processes that are at the base of the measured parameters. In the first curve, scatter, fluorescence, phosphorescence, and the internal conversions that heat a substrate are uppermost, while in the third curve complex biochemical processes are modified by the input of exogenous energy. The second curve is really a modification of the first that takes into consideration the energy source. It has to be admitted, however, that these curves suffer a limitation for straightforward comparison that is discussed later: The absorption spectrum was determined in vivo, whereas the other two curves were measured in vitro, and melanin probably changes considerably during extraction from the eye.[2]

Light that is scattered in a system can be regarded as energy that has had its direction of propagation redirected and that does not take part in influencing the substrate. In fluorescence and phosphorescence the incident energy is, in effect, stored momentarily before being emitted again at a different wavelength. These three processes can sometimes be harnessed for useful ends (e.g., fluorescence angiography), but they are the origin of many problems in optical systems. The internal conversions represent the ever-present thermodynamic requirement for entropy increase, but they can be used if it is desirable to heat the substrate, as, for example, in making thermal lesions in the retina for therapeutic purposes.

The remaining parameter that determines the total energy dose is exposure duration, discussed more fully later.

DETERMINING THE RETINAL EXPOSURE

When considering how much radiation reaches the retina. a number of obvious but often overlooked factors must not be forgotten. These include the direction of gaze, whether the pupil is obscured by the lids, the pupil diameter, and whether the subject has "screwed upthe eyes or responded with an aversion reflex. Any or all of these factors may have to be included in an exposure calculation.

The effects of retinal exposure also depend on the optical properties of the preceding media and how they transmit radiation as well as on the chromophores in the retina itself. The situation is, in fact, a complex one. Not only does the neural retina have its own chromophores, but the other layered structures associated with it do so as

well. It is necessary, therefore, when considering the retina to take account of the ocular media and these other layers and the energy that they trap. This is because this energy can either spread to the retina proper as a thermal insult or, by compromising the function of closely related tissues, compromise the retina too by upsetting normal physiologic processes. The chromophores in the retina and the retinal pigment epithelium (RPE), together with the melanin in the RPE and choroid and the hemoglobin in the choriocapillaris, must be included in any discussion of radiation insult to the retina.

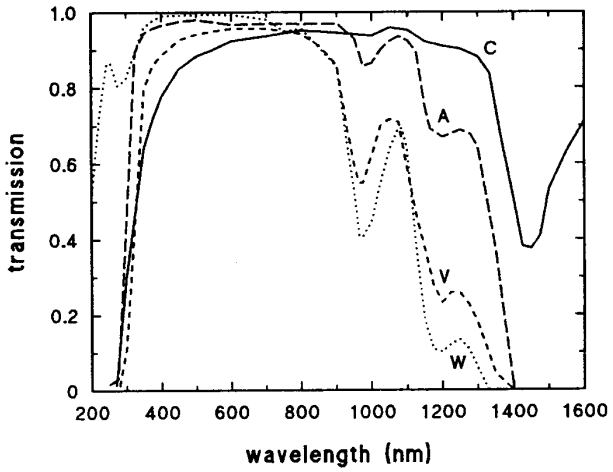

Figure 116-3. Spectral transmission curves of rhesus monkey ocular media. C, Cornea (path length = 0.55 mm),. A aqueous (path length 2.86 mm); V, vitreous (path length = 11.55 mm); and, for comparison, the dotted curve, W, of distilled water (path length = 18.5 mm). (Data from Maher.[11])

Of the pre-retinal ocular media, only the lens has significant absorption, scatter, or fluorescence in the region of the spectrum from UVA to IRC. Figure 1163 shows absorption curves for the cornea, aqueous, and vitreous; the extent of absorption changes with age in these media, but only significantly in the cornea at the shorter wavelengths. Also shown is the curve for water, which obviously determines most of the absorption of these media. the effect on the passage of radiation to the retina of these media is slight, except for the lens, and occasions little disagreement among published authorities. Obviously, in aphakic eyes or those with intraocular lens (IOL) implants the situation is very different, and in the former eves visibility of UV light does diminish with age. In those with IOLs the material of the lens controls the quality of radiation that reaches the retina. These lenses are usually made of polymethyimethacrylate (PMMA) and may have a UV blocker, and perhaps pigmentation, to mimic the transmission properties of an aging crystalline lens.[3]

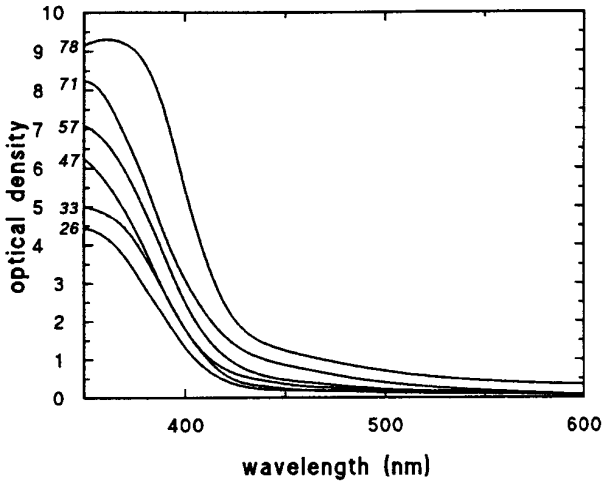

Figure 116-4. Spectral curves for the optical density at the thickest part of the lens (pole) for six typical lenses taken from the study by Mellerio.[11] The age of each lens is shown in italic figures on the ordinate at the start of each curve.

The crystalline is well known for its absorption of UV and blue light but there is in the literature a considerable range of transmission values.[4] Figure 116-4 shows transmission curves for six lenses of different ages, but authors have not always specified the age of their material or whether it was in any way cataractous. Figure 116-5 shows results at one wavelength (400 nm) from nine studies that do show the effects of aging. The very large discrepancies -well over 1 log unit at 400 nm - are thought to derive partly from a real variation of optical properties, scatter as well as absorption,' and partly from the various techniques of measurement. Weale[6] criticizes Werner[7] for the improper inclusion of some of the data in his figures, which are, nevertheless, included in Figure 116-5. The methods of determining lens absorption range from subjective color matching or photography of the crystalline *in vivo* to various ways of dealing with lenses taken out of the eye. Another difficulty arises because the loss of light transmission in the lens is due to scattering (or fluorescence) and not only to absorption.[5, 6] It is necessary to bear this in mind when using lens transmission figures, as the method of measuring them may have influenced their magnitude. At short wavelengths and especially with older lenses, the spread of lens transmission values is so great that individual

cases can readily be found that would support the choice of almost any figure for transmission-you pay your money and you take your choice.

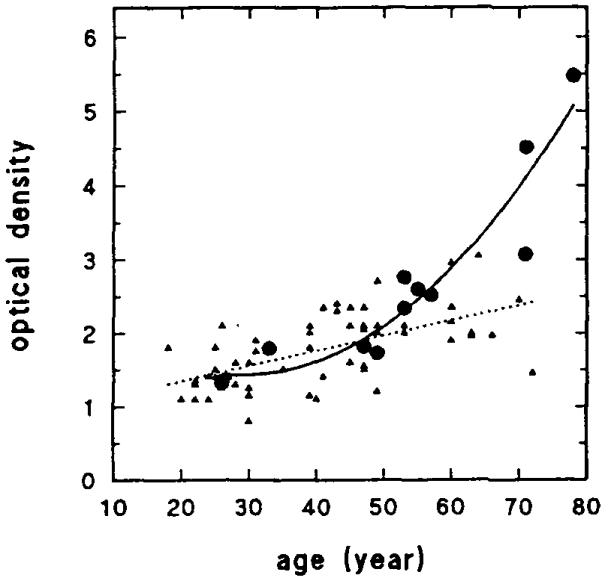

Figure 116-5. Optical density of human lenses at 400 rim plotted against age. The large filled circles and the fitted second-order polynomial curve are from the paper by Mellerio[72] and the small solid triangles are from Werner,[7] who took the data from eight other authors for comparison. Werner's data are fitted by a straight line regression (D = 0.021 age + 0.94). The two sets of data clearly show the increase of density with age and the increase in range of values that accompanies this.

Reflection at, and light scatter within, the layers of the neural retina, RPE, and choroid are not usually considered important in assessing the possibility of retinal light damage, because the fraction distributed away from the retina in this way is small compared with the direct irradiation within the retinal image (typically 10 to 15 percent but see reference 8). The various chromophores in these layers absorb light, which may initiate damaging processes, and thus modify it before it is passed on to deeper chromophores. A complete picture of what happens in these layers may be complex, and it is necessary to consider the known chromophores in turn to gain an understanding of the processes involved. Again, it must be remembered that absorption spectra may not adequately describe the possibility of damage, because they are not action spectra. Indeed, those action spectra that are currently available for light-induced damage in the retina do not all closely match the absorption curves of the most obvious chromophores. Nevertheless, it is usual to consider those discussed below as the prime movers of damage.

The yellow macular pigment xanthophyll is confined to the outer plexiform layer; its absorption spectrum is shown in Figure 116-6. If this pigment is a damage originator, it might be involved in the photochemical damage that is described below. Its contribution to thermal damage might be small because its total absorption is much less than that of melanin, but in the fovea even small lesions can have enormous visual effects.

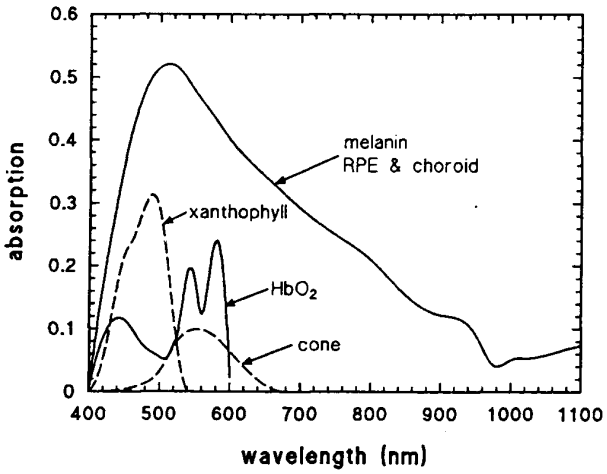

Figure 116-6. Spectral absorption curves for melanin, xanthophyll, cone pigments, and oxygenated hemoglobin. The absorption is the fraction of incident radiation at the cornea that is absorbed in the pigment-bearing structure in the eye. Consequently, the curves include absorption in the lenticular pigment: the age of the lens chosen for the calculations was 52 years.[72] The cone pigment absorption is calculated for a cone of average outer segment length and pigment loading. The hernoglobin is assumed to be in a retinal vessel 10 μm in diameter and the macular pigment uses the values found in whole retinal preparations by Marshall (personal communication). The melanin figures are for the combined absorption in the RPE and choroid and are derived from unpublished data from Marshall and the paper by Boulton and coworkers.[13]

The visual pigments are also obvious chromophores that could originate damage. Their total optical density is small, and their primary involvement in thermal damage is not likely to be extensive or, in clinical situations, important, except in the fovea. Undoubtedly, they make a contribution to photochemical damage and their absorption spectra (Fig. 116-6; see also Fig. 11614) are

not incompatible with the presumptive action spectra for one type of this damage. For example, Harwerth and Sperling[9, 10] were able to produce damage in blue cones or green cones by exposing primates to appropriately colored lights. However, the fact that the blue cones had a lower threshold than that of the green (and presumably the red) does not correlate with the total quantity of pigment present in the outer segments of each cone type. Also, it turns out that cones are more sensitive than rods, although more rhodopsin is present in the longer outer segments of the latter. This situation has little to do with pigment absorption and arises because the repair processes within the cones act more slowly to restore normality than those in the rods.

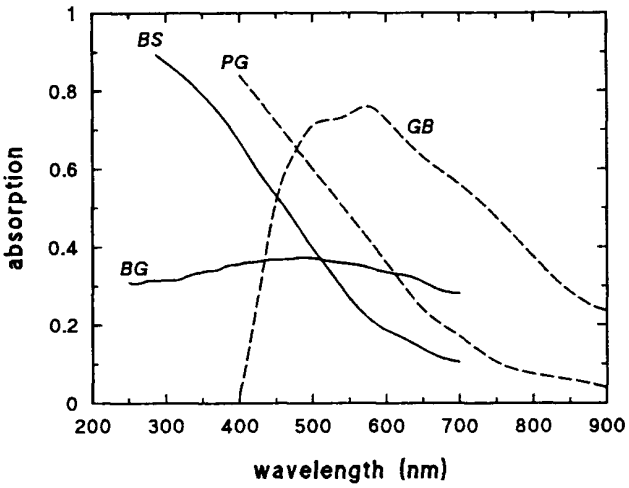

Figure 116-7. Spectral absorption curves of melanin in various situations. *BG,* a suspension of human melanin granules pooled from the RPE of eyes over 50 years old; suspension in buffered saline. *BS,* a solution of human melanin granules as in *BG,* solvent was 1M KOH. *PG,* mean results from 16 flat mounts of human RPE measured under a microscope; age not specified. *GB,* mean results of 28 human retinas, RPE, and choroid, calculated to include the preretinal ocular media (which explains the short-wave length cutoff); age of donors ranged from 23 to 78 years. (Data from: *BG* and *BS*[13]; *PG*[17]; *GB*[8].)

Hemoglobin is present in the internal retinal circulation and is often a target for clinicians trying to close off or destroy blood vessels and circulatory anomalies. Usually, an immediate effect is required, so the intention is to produce a thermal lesion by focusing radiation on the offending vasculature. There is also a "lake" of hemoglobin in the choriocapillaris. The absorption of this blood pigment is, therefore, held to be important; Figure 116~6 shows this for the oxygenated form. The expectation that yellow light from a dye laser should be used to destroy blood-bearing structures has been only partially established because in total there is not much absorbing power in the thin layers of blood that retinal or choroidal capillaries contain. In addition, the flow of blood in retinal vessels carries away heat, so that it is difficult to close a vessel without spreading energy to neighboring structures.[11] Blood flow is not an important factor for the choriocapillaris, because, as Birngruber and coworkers[12] showed, threshold doses for thermal lesions of the retina are not affected by the presence or absence of choroidal blood flow.

Undoubtedly, the most avidly absorbing chromophore present is the melanin of the RPE and choroid. Figure 116-7 depicts the spectral absorption curve for a solution of melanin; it is not possible to measure absorption for an individual granule, as it is so great. The absorption of the solution is believed by some.[13, 14] to describe adequately melanin absorption in the eye, but the conditions used to obtain solution (hot, concentrated potassium hydroxide [KOH]) are so harsh and unphysiologic that it is difficult to believe that melanin solution can tell us much that is of use for understanding the situation in vivo. Spectral absorption can be measured for suspensions of granules (Fig. 116-7), but the sieve effect[15] gives a curve that is too flat. In addition, scattering of light among the granules leads to artifactual wavelength-dependent effects. The curves of Boulton and coworkers[13] show minor peaks and troughs in the plots for melanin granule suspensions; these may be due to selective scatter or to contaminants that do not find their way into alkali solution.

The relative absorbed spectral dose cannot be calculated without knowing the spectral absorption of melanin. So, if solutions yield values that are doubtful and one is suspicious of suspensions, might it be possible to measure absorption of melanin in vivo or in vitro? Several reports have raised such questions, and not only in ocular circles,[16] and though some have attempted measurements in vitro, to date none has convincingly measured melanin absorption in the living eye. Geeraets and Berry[8] were the first to measure absorption of the RPE and choroid in human tissue (Fig. 116-7), but this measurement is expressed in terms of light incident at the cornea and so includes the transmission properties of the ocular media. Also shown is the curve Gabel and colleagues[17] made using a microscope to examine pieces of human RPE sheet mounted on a slide. The solution and the tissue curves have approximately the same slope, but in linear plots this signifies little: Curves such as these speak of considerable variation between studies that arise either from the different techniques used to extract and measure

melanin absorption or, more interestingly, from differences in the melanin itself, e.g., those due to genetic factors. Tissue curves such as those in Figure 116-7 give the mean picture, but as the melanin is distributed in granules they are not useful when considering retinal heating with microscale models.

EXPERIMENTAL LIGHT DAMAGE TO THE RETINA

The damage that radiation produces is usually classified under one of three heads (Fig. 116-8). In reality, there is a continuum of observable effects, and the three divisions are more for convenience of discussion than they are theoretically significant. The intermediate classes of thermally assisted chemical effects and thermoacoustic effects underline the seamless nature of the continuum.

Photochemical Damage

By definition, photochemical damage is brought about by photons of such high intrinsic energy (i.e., short wavelength) that chemical bonds of the molecules that compose the tissues are directly split. Once a bond is broken in a biomolecule the molecule may change its conformation and thus lose its function. For example, a cross-linking bond in a protein may be split, allowing the molecule to uncoil and change shape dramatically. Of course, with such complex molecules as proteins there may be no conformational change if one or even a few bonds are broken, but whether this happens depends very much on which protein is under consideration. The evolutionary significance of neutral mutations, some of which result from photochemical damage to DNA, has a bearing on the ineffectiveness of photochemical damage, as may be argued from the views set forth by Jukes.[18]

The portion of the molecule, or the functional group, that absorbs the incident photon is termed a chromophore. The electronic transition to the excited state absorbs the energy of the photon, but the decay and release of this energy may proceed by a number of routes. One may be the transfer of the energy to split a bond either in the chromophore or elsewhere in the molecule, even sometimes at sites quite remote from the absorption locus. If a covalent bond is split, each fraction of the molecule may retain one of the shared electrons to become a free radical. These radicals are highly reactive and exist only briefly before they react with neighboring molecules.[19]

If the energy in the incident photon can be coupled to the molecule more efficiently, the likelihood of bond splitting is enhanced. This may be done with a vital dye, which is an efficient chromophore that can pass on its trapped energy to the bound molecule, thus leading to bond breaking. Such vital stains as toluidine blue, rose bengal, hematoporphyrin, and many others can so increase the energy trapped that irradiated cells containing them may die.[20] This process is used therapeutically to treat certain tumors in the eye and elsewhere.[21] Vital stains are exogenous sensitizers. There is a range of endogenous sensitizers too: chlorophyll, riboflavin, and bilirubin are three that have been suggested to have biologic importance.[22] Lipcifuscin, which is found in increasing amounts in the aging retina, might also act as a chromophorell in elderly eyes, in which age-related macular degeneration is often a problem. Interestingly, the visual pigment retinol can act as a sensitizer.

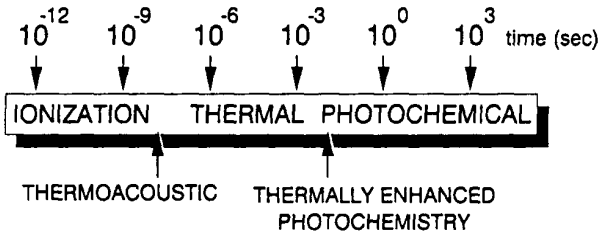

Figure 116-8. The mechanisms for light-induced retinal damage related to exposure duration.

The formation of free radicals is undoubtedly more important in producing tissue damage than the splitting of bonds. Free radicals, such as singlet oxygen, can attack many molecule types and render them ineffective. It is this hyperactivity that makes the free radicals so toxic.[24] There is an additional risk in tissues where there is a large concentration of cell membranes, which are, of course, composed largely of lipids. Once one of the $-CH_2-$ groups in the chain of a polyunsaturated fatty acid in a lipid reacts with a free radical, a chain reaction of lipid peroxidation starts. and it ends eventually in the decomposition of the molecule. As lipids form the backbone of membranes, the membranes are broken down. This process is seen in Figure 116-9, which shows the early stages of light damage in the outer segment of a pigeon cone.[25] The retina is thus well placed to sustain this kind of damage, as it has an endogenous sensitizer and an abundant supply of oxygen for singlet oxygen formation and it is nicely placed under a transparent focusing system designed especially to collect light.[26, 27] Its most important structures. the membrane discs of exposure to a bank of standard fluorescent lamps that were as bright as an overcast London sky. Note the punctate damage to the disc membranes resulting from

the early stages of light damage induced by lipid peroxidation the outer segments of the photoreceptors. are made almost entirely of lipid just waiting to be peroxidized.

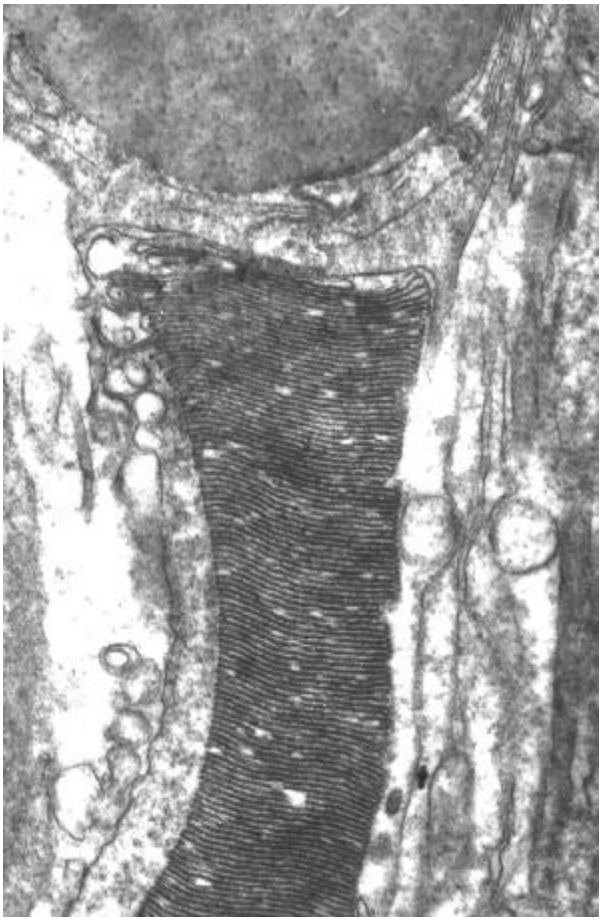

Figure 116-9. Electron micrograph of part of the outer segment of a pigeon cone 7 days after several hours' .

Whether a bond is broken depends on the energy of the incident photons: If the energy is not great enough, the bond will never be parted. The probability that the bond will be broken depends on the number of photons incident on a chromophore, provided that they have sufficient intrinsic energy. The changes that a particular wavelength of radiation brings about are always the same, but they proceed at a rate that varies only with the incident flux. Thus, photochemical reactions are dose dependent: A certain number of photons are needed to complete the reaction by breaking a certain number of bonds, and the reaction proceeds at a rate determined by the rate at which photons arrive, that is, by the irradiance. Consequently, a reciprocal relationship exists between irradiance and exposure duration (Fig. 116-10). Indeed, the demonstration of a direct reciprocal relation between these two quantities is usually taken as confirmation that a process is photochemical.

Figure 116-10 shows that two distinct processes can cause damage to the retina. One has the expected slope of - 1, establishing this as photochemical; the other has a very different and much smaller slope, representing thermal damaging processes (see further on). There is a wave le ngth-depende nt effect; the argon laser, with its energetic photons, readily causes photochemical damage, whereas the infrared Nd:YAG laser, with photons only half as energetic as those from the argon device, causes only thermal damage.

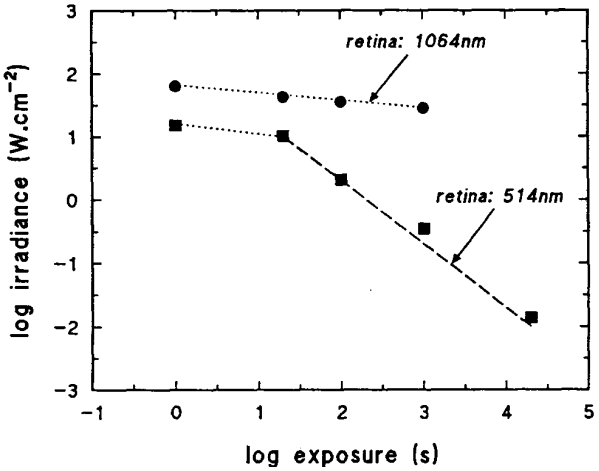

Figure 116-10. Log retinal irradiance (as measured at the cornea) plotted against log exposure duration for production of just visible threshold retinal damage from single exposures to argon laser radiation (514 nm, ■) and to Nd:YAG laser radiation (1064 nm, ●). The dashed line has a slope of - 1 and is a by-eye fit: it clearly demonstrates the reciprocal nature of the damage at longer exposures for argon radiation. The dotted lines are byeye fits of very low slope and represent damage induced by thermal processes. These data are selected from Sliney.[21]

Photochemical damage, of which sunburn is a good example, usually demonstrates delayed onset. In the retina the delay may be several minutes or several hours. This is because overall cellular processes have turnover cycles of minutes or hours and photochemical damage works by destroying perhaps just one molecular component of a cell's biochemistry. In addition, photochemical damage to the retina produced in laboratory studies is often widely distributed and diffuse. as would be expected if the chromophore were some endogenous component of a basic biochemical process, such as

glycolysis or cell respiration. There is a second reason for the widespread nature of this damage in the retina. As the irradiances are low and the exposures are long, the light sources used are often extended (e.g., fluorescent lamps). In extralaboratory situations, damage may result from, for example, reflection from snow, and this, too, represents an extended light source.

It has become customary in recent years to classify retinal photochemical damage as either type 1 or type 2. This is an unfortunate choice of name, however, for it leads to confusion with the terms generally used in photochemistry for reactions in which the photosensitized molecule reacts either directly with the substrate (type 1 reaction) or with molecular oxygen to form singlet oxygen or superoxide free radicals, which in turn react with the substrate (type 2 reaction). To avoid this confusion, we prefer the eponymous *Noell-* and *Ham-* type damage, to acknowledge the respective discoverers of these phenomena. The classification is based on damage distribution within the retinal layers and on the exposure parameters, but the division is not wholly satisfactory. Some authorities have found the division artificial, and recent evidence points to several damage mechanisms that overlap to produce a continuum of effects. from Noell to Ham damage. Nevertheless, consideration of photochemical retinal damage can well begin with a discussion of these two classes of damage; they are described here together with some criticisms. It must also be remembered that once a damage process is under way, the end results are often very similar. Therefore, workers have had to pay particular attention to damage caused by just suprathreshold doses of radiation. This makes the work difficult and very dependent on subjective evaluation of histologic material.

NOELL DAMAGE

Noell damage is caused by long exposures (typically hours or even days) to low irradiances; at threshold, the first manifestations of damage are seen in the photoreceptors. The area of retina damaged is usually large, for the reasons mentioned earlier. The first report of this type of damage was by Noell and coworkers,[28] who showed damage in the retinas of rats in cages surrounded by fluorescent lamps. It was soon established that cones were more sensitive than rods to light damage, which initially is intriguing but is now explicable in terms of photoreceptor repair processes (see later). By exposing animals to colored lights it is possible to damage the group of cones sensitive to these colors,[25] and even to produce animals that are effectively colorblind.[10] It is also possible that Noell damage is responsible for the reduction in blue light sensitivity that develops in ophthalmic surgeons who have been cumulatively exposed to argon laser radiation for many hours when treating patients in the laser clinic.[29] If the damage appears first in the photoreceptors, and cone populations can be selectively destroyed, the chromophore responsible might be in the receptor outer segments. The finding that the action spectrum of Noell damage corresponds relatively well with the absorption spectrum of the visual pigments supports this claim.[28,30-32]

HAM DAMAGE

Ham damage is caused by irradiances higher than those that produce Noell damage, and the exposures are shorter (seconds to minutes). The area of retinal damage is smaller because the irradiances required are usually possible only with light sources that are presented to the eye in collimated beams. The damage. first described by Ham and colleagues,[33] is held to originate in the RPE. Ham measured the action spectrum of the damage[34] and showed that its sensitivity increased with reducing wavelength of light (Fig. 116-11). Consequently, this Ham-type damage is sometimes known as Blue Light Damage. In earlier papers, Ham's group[34] thought that the site of origin of the damage was “located in the outer segment of the photoreceptor and perhaps in the pigment epithelium.” Later, they, were able to show that the greatest damage appeared in the RPE but that the photoreceptor was also affected.[26] Because of the involvement of the RPE, melanin has been suggested as the chromophore responsible, but as Figure 116-11 shows, the blue light damage action spectrum does not remotely match the melanin action spectrum for uptake of oxygen (see Fig. 116-2) or the absorption spectrum for melanin solution.[14] Since the Ham action spectrum was made for a whole eye and the absorption of the ocular media is included in the curve, the disparity would be even greater for direct retinal exposure.

Sarna[2] reviewed the role of melanin as a protector of cell integrity. It is established that melanin protects the cells containing it from oxidative damage-the RPE and choroidal cytoplasm have very high oxygen concentrations-probably by binding oxidative catalytic metal ions, which are thus removed from the biochemical arena of the cell. Sarna also reviewed the evidence for a toxic role for melanin, which has frequently been suggested, but concluded that in vivo, as opposed to *in vitro*, melanin would not normally release free radicals or promote oxidative processes. With aging the constant exposure to high oxygen levels and years of daily light doses might cause the antioxidant properties of melanin to diminish and allow its pro-oxidative proclivities to gain the upper hand, so inducing the damage we know as age-related macular degeneration. Sarna's conclusions would seem to support the doubts that melanin is the chromophore that

initiates Ham-type damage in the retina.

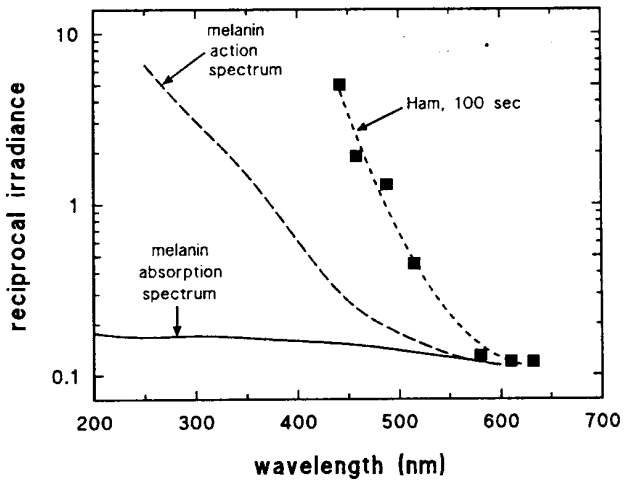

Figure 116-11. The blue light action spectrum from Ham and coworkers[34] for 100 sec exposures of monkey eyes; the ordinate (note the logarithmic scale) is reciprocal retinal irradiance (cm2/W) for just fundoscopically visible lesions 24 hours after exposure; the data points are shown fitted by a second-order polynomial curve. Shown for comparison are the melanin action spectrum for oxygen uptake and melanin solution absorption curve (see Fig. 116-2 for details)[14]; both curves have been shifted vertically to be coincident with the Ham curve at wavelengths above 600 nm; for both these curves the ordinate units must be considered arbitrary.

Though the existence of blue light damage has been confirmed by several reports and the data have been incorporated into the codes of practice for the safe use of intense light sources, accounts of where the damage originates do not completely agree. The view of Ham and coworkers[26] that it is not easy to distinguish between primary damage to the photoreceptors and secondary damage resulting from damage to the RPE must be given due weight. Ham has suggested[34] that blue light damage probably is not the result of a single process, and the literature supports this view. Rapp and associates[31] have suggested that mitochondrial enzymes may be the chromophore for the blue light damage and cite the initial swelling of these organelles to substantiate their claim. Pautler and coworkers[35] also implicate mitochondria, and in particular the cytochrome enzymes resident therein. Using electron microscopy they showed that the initial damage resulting from light exposure was to the mitochondria. Kremers and van Norren[36] suggest that the products of photobleaching may constitute the chromophore. Whatever the chromophore, Ham and coworkers[26] have shown that oxygen seems to be necessary for Ham damage to occur and that if oxygen tensions are increased in the retinas of irradiated animals, the damage increases and the threshold drops. They also demonstrated that certain free radical quenchers protected the retina from the effects of increased oxygen tension. Thus Ham damage would appear to involve free radicals, especially oxygen.

Kremers and van Norren[36] pointed out that most laboratory studies that show Noell damage have used rodents exposed for a long time, whereas studies that have reported Ham damage have been made with primates exposed for up to 4 hours. Reasoning that this division of species and exposure parameters might explain some or all of the differences between Noell and Ham damage, they exposed primate retinas for as long as 12 hours. They then compared their data with those from the literature by plotting exposure duration against the damage threshold irradiance for both types of damage.[37] Their graph is replotted as irradiance against exposure in Figure 116-12, which convincingly shows a bipartite curve that separates the two damage types. The Ham region shows a reciprocal relation between irradiance and duration, with the expected photochemical slope of - 1. There is an abrupt change at 12 hours, when the threshold drops by a factor of 100 before Noell damage continues for longer periods, also with a slope of - 1.

Kremers and van Norren's examination of the data shows no obvious reason for a correlation between animal species and type of damage except that it is difficult and more expensive to expose primate retinas continuously to light. Their own extended exposures of monkeys suggested that the correlation of damage type with species was fortuitous. The report of Rapp and coworkers[31] confirms this view: They claim to have produced in one animal type, the Long-Evans rat, light damage due to two different mechanisms initiated by UVA and green radiation. One was initiated by UVA radiation peaking around 355 nim and 500 $\mu W.cm^{-2}$ at the cornea and revealed by minimal disorganization of the photoreceptor outer segments after 1 day, pyknosis of their nuclei, and severe swelling of the rod inner segments and RPE mitochondria. Exposure to 5000 $\mu W.cm^{-2}$ green light, on the other hand, caused severe derangement of the inner and outer segments but left the RPE appearing normal. The intensities of the radiation for threshold damage are markedly different at the cornea, and this difference increases greatly for retinal intensities if the high absorption of the ocular media to UVA radiation is taken into account. As might be expected, the UVA radiation was also markedly inferior in bleaching rhodopsin compared with the green light.

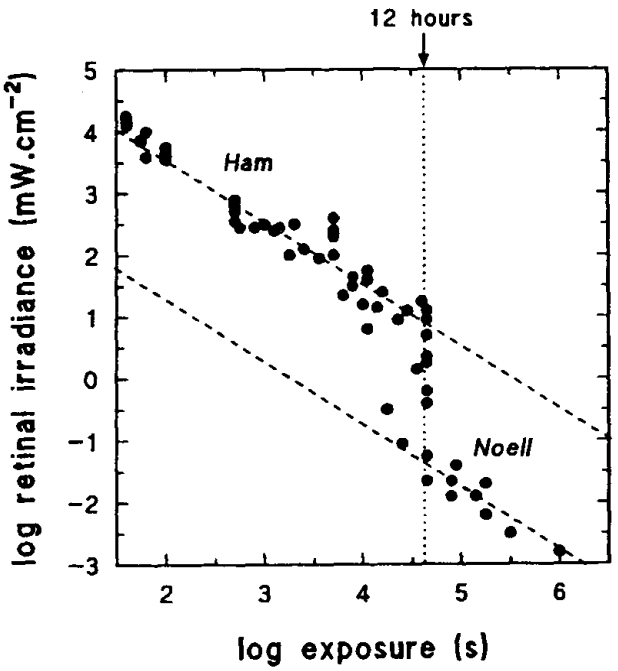

Figure 116-12. Log retinal irradiance plotted against log exposure duration forjust detectable retinal photochemical damage. The data are taken from Kremers and van Norren,[37] who derived them from the literature of photochemical damage to different species of animal and different exposure regimens. The arrow indicates exposure of 12 hours, and the dashed lines have a slope of - 1.

Van Norren and Schellekens[32] have also producedNoell and Ham damage in rats. They confirm that the action spectrum of Ham damage in the rat is like Ham's monkey blue light spectrum, but they do not know what pigment or pigments initiate the process (Fig. 116-13). Pautler and associates[35] measured an action spectrum for reduction of the transepithelial potential in bovine RPE in an Ussing chamber. They argue that this spectrum fits very well the absorption spectrum of cytochrome C oxidase, which is found in mitochondria, the organelles they believe to be the initial site of Ham damage. Their action spectrum is also shown in Figure 116-13: In their paper they claim that it fits well with the action spectrum from Ham's group[34]-between 400 and 500 nm-but the fit is not too convincing. They are on surer ground when they point out the considerable disparity at shorter wavelengths and, as a result, suggest that two mechanisms must be responsible for the damage, one dependent on blue light and one on UVA radiation. Some chromophore with higher absorption in the UVA region is therefore required, and in Figure 4 of their paper they compare the absorption spectrum of all-*trans* retinol (which peaks at 360 to 370 nm) with their action spectrum and reject it as a candidate for damage initiation in favor of cytochrome C oxidase. This is reasonable, because their preparation contains only the RPE separated from the neuroretina an hour or more before; for an intact retina, the basis of Ham's group[14] experiments, all-*trans* retinol and other products of bleaching are formed continuously during exposure. This line of argument could be held to support the Kremers and van Norren model described below, but a fuller examination of the absorption properties of the various bleaching products should be undertaken, together with a consideration of the contribution of each that will ensue from the kind of dynamic equilibrium between bleaching and regeneration that this model requires for the longish exposures that produce Ham damage.

Because the action spectrum of Noell damage fits the absorption curve of the visual pigments reasonably well,[28, 38] van Norren and colleagues[39] proposed an explanation for their unifying curve. With low irradiances the most sensitive damage-inducing chromophores, the visual pigments, couple energy to a photochemical damage mechanism (probably lipid peroxidation of disc membrane[25, 27]), and Noell damage processes are set in train. With higher irradiances the visual pigments are rapidly bleached and effectively removed, so that a less sensitive damage-inducing chromophore, nature unknown, couples energy to RPE systems, which results in Ham damage. This scheme might have two versions (Fig. 116-14). In version A, more intense radiation bleaches the visual pigment to such an extent that radiation can reach an unspecified sensitizer, presumably in the RPE. In version B. it is the products of bleaching themselves that are the sensitizer: The bleach-regeneration cycle of visual pigments uses several different products in dynamic equilibrium within the photoreceptor and a retinol store in the RPE. The UVA absorption of retinol in the RPE[35] (discussed earlier), coupled to that of mitochondrial enzymes, might explain the origin of Ham damage.

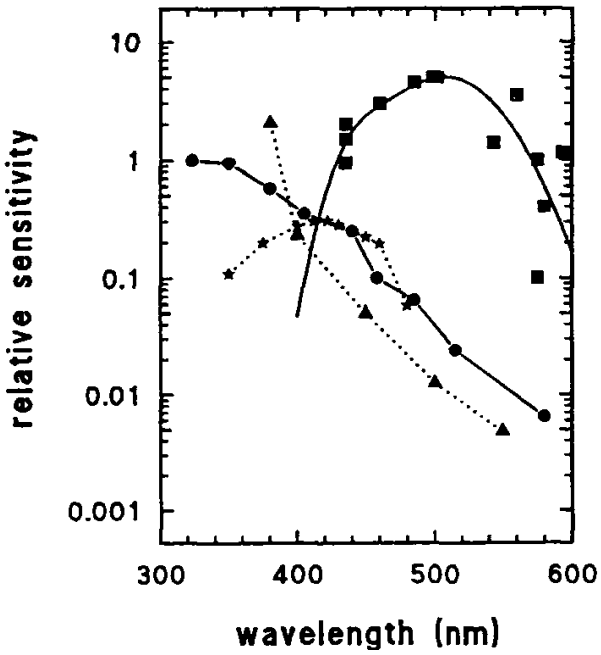

Figure 116-13. Action spectra for Noell and Ham photochemical damage to retina. ●, Spectrum for Noell damage, data points from Noell and coworker[28] and Williams and Howell,[38] and the line is the scotopic visibility curve. ■, Spectrum for 100 s exposure of monkey from fiam and coworkers. ▲, Spectrum for rat damage from van Norren and Schellekens. [32] ★, Spectrum for bovine RPE transepithelial potential from Pautler and coworkers.[35] The spectra have been moved vertically by arbitrary amounts for comparison.

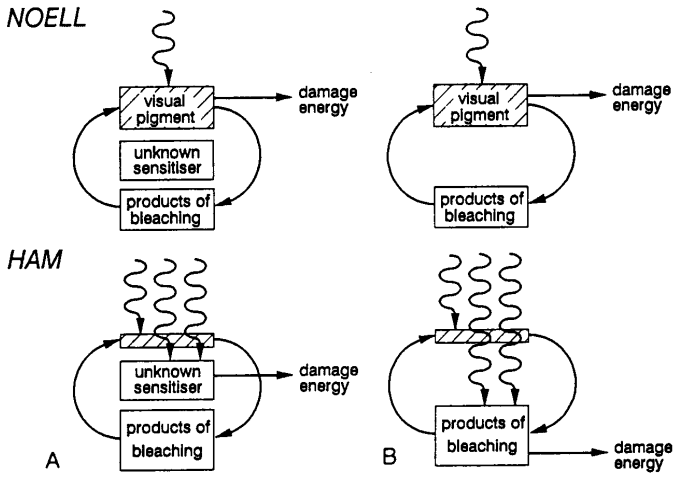

Figure 116-14. A summary diagram of two possible versions of the Kremers and van Norren explanation of photochemical damage to the retina. In Noell damage the irradiance is low and a dynamic equilibrium is established between the bleaching of visual pigments (hatched box) and their regeneration: energy is coupled from the visual pigments acting as sensitizers to the damaging processes. For flam damage the irradiance is high and the equilibrium of bleached products and regenerated visual pigment is pushed so that hardly any pigment is present: in & the radiation can pass to the as yet unknown sensitizer to couple energy to the damaging processes, whilst in B, the products of bleaching are suggested to be the sensitizer.

As photochemical reactions depend on the energy of the photons and the rate of reaction depends on the number of photons incident per unit time, there is no "threshold" for a photochemical reaction. It simply happens faster or slower depending on the irradiance. That threshold criteria were used by the various investigators whose data were plotted by Ki-emers and van Norren might, therefore, seem confusing, as the effects are photochemical. The fact is that thresholds arise here because the effects have to be of a certain magnitude before they can be detected. Mostly. ophthalmoscopic visibility has been taken as the threshold criterion for retinal lesions, so the concept of threshold has significance only at a macroscopic level.

Photochemical damage is not immediately obvious because the damaged molecules may take part in biochemical pathways that have finite rates of reaction, so lack of products or excess of intermediates takes time to be noticed. Also, all cells have repair and renewal processes that attempt to nullify any damage. Of course, if the incident radiation destroys molecules faster than they can be repaired, there will be a net increase in damage; the effect will he seen subsequently and a threshold established.

At normal body temperatures, thermal processes constantly denature biomolecules. but an equilibrium exists between thermal degradation and repair processes in a healthy cell. There is always spare capacity in the repair processes to provide for any emergency situation, but elevation of body temperature increases the rate of degradation and reduces the spare repair capacity. This explains the familiar observation that at high body temperatures photochemical damage is enhanced and the irradiance -thresholds- are lowered.

The presence of repair processes leads to a departure of photochemical damage thresholds from the strict reciprocity relation that is expected.[40] This will be especially obvious at long-duration exposures. in which the irradiances are low and the repair processes very nearly balance the damage mechanisms. This concept is illustrated in Figure 116-15, in which the ordinate represents the irradiance, which is directly related to the rate of degradation, and the abscissa is the duration of exposure. The area in which the rate of repair can exceed the rate of degradation is shaded; the solid line shows the expected relation between irradiance and duration departing from strict reciprocity at shorter exposures to an asymptote parallel to the time axis at longer times.

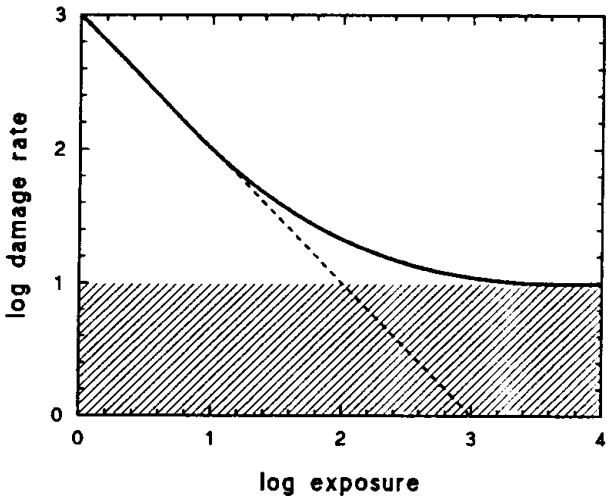

Figure 116-15. Damage rate (or irradiance) plotted against duration of exposure for photochemical retinal damage (both axes in arbitrary units). The shaded area represents the area where repair processes exceed the rate of degradation, the dashed line shows the reciprocity relation of slope - 1, and the solid line the practical division between damage and no damage.

Thermal Damage

The term -thermal damage- implies a rise in temperature within the tissues that are damaged: To understand the process it is necessary to know what is meant by temperature rise and how incident energy can bring this about. Temperature is a measure of the kinetic energy stored throughout a large population of molecules in their vibrational, rotational. and intermolecular motions-it has no meaning for individual or very small assemblages of molecules . An analogy to temperature would he the amount of jostling in a crowd at a football match, a concept that becomes meaningless for a very few individuals in an otherwise empty stadium. If the temperature or stored kinetic energy increases, there comes a point when molecules shake themselves free of the influence of other molecules (breaking intermolecular bonds) or even shake themselves to pieces (breaking intramolecular bonds). This energy comes initially (1) from internal conversions, where excited electronic states decay, releasing energy to vibrational or rotational states; or (2) by direct absorption of incident energy into vibration or rotation. It is also possible to have transfer of energy from a variety of processes through the members of a population of molecules.

The kinetic energy that is temperature comes, as far as this discussions concerned, from the input of radiant energy into the retina. Unlike photochemical reactions, in which there is no irradiance threshold and irradiance and exposure duration are linked by reciprocity, thermal reactions do have irradiance thresholds and do not show reciprocity. This is because there is no photon energy requirement for bond breaking. Sufficient energy to increase the temperature can come by any route. while at the same time this energy can leak harmlessly away, especially if the exposure duration is long. Consequently, the energy required to produce a thermal effect increases with exposure time and the irradiance-duration relation for just detectable lesions is not linear but is typically of the form:

$$E_e = K \cdot t^{-0.25} \qquad (7)$$

where K is a constant and t is between 20 μsec and 10 sec.[22, 40] In Figure 116-10 the "thermal" portions of the plots have slopes of about - 0.12, so for the particular experimental circumstances that apply to this figure the exponent is about half the usually accepted value.

The major structural molecules of biologic tissues are proteins, which are held in highly specific configurations by hydrogen bonds and Van der Waals' forces. These important cross-links are relatively weak and readily destroyed by heating. When the bonds are broken, the protein may uncoil and recoil into a new

configuration, which could completely remove any useful function that the protein had, This is the process of denaturation or coagulation and is easily seen when poaching or boiling an egg. The unfolding and refolding takes a finite time that may be about the same as the duration of some of the shorter laser pulses that can induce thermal damage. Proteins are particularly sensitive to heat, and they turn out to be the cell's Achilles' heel for thermal damage. If even a few protein molecules are destroyed by heat, the repair processes may not be able to effect a repair and cell death could follow. Marshall[41] has argued that only a small portion of a cell's total protein complement need be damaged for cell death to occur. In such a highly specialized tissue as the retina, damage that leads to cell death in any layer is readily spread to other cells whose existence depends on the functional integrity of the first cells. A typical situation occurs when photoreceptors are damaged by any type of insult and complete retinal atrophy ensues. For radiation damage in the retina this process can be termed damage amplification, for it is only necessary to destroy a few pigment epithelial cells or photoreceptors to induce full-thickness retinal atrophy.

Thermal effects can occur only if incident energy is trapped or absorbed in the substrate molecules, This absorption is easily measured, and it is generally expected that an absorption value of a substrate will provide an indication of what increase in temperature might result. There is thus considerable interest in the absorbing properties of ocular tissues on the part of investigators trying to evaluate what effects are produced or trying to produce desired treatment schemes. Spectral absorption curves may yield a full specification for a substrate, but if the radiation comes from a wavelength-limited source, such as a laser with its monochromatic output, it is often more convenient to specify the absorption at the chosen wavelength. This absorption is related to the incident radiation by the relation

$$T + A + S + R = 1 \qquad (8)$$

where T is the fraction of radiation transmitted through the substrate, R is the fraction reflected, S is the amount scattered out of the substrate, and A is the fraction absorbed. The figure of A or T by itself is not always particularly informative, and the concept of penetration depth is sometimes used. This is defined as the distance, d, through a substrate where the intensity of the radiation has fallen to l/e of its value at the surface; so

$$\frac{I_t}{I_o} = \frac{1}{e} \qquad (9)$$

where I_o is the incident radiation intensity and I_t is the intensity of the transmitted radiation. The penetration length d is particularly useful when it is necessary to consider how deep into a substrate the radiation is deposited.

There are a number of specific chromophores that occur in the retina and that readily absorb light. Figure 116-6 shows the spectral absorption curves of the four thought to be most important for inducing thermal effects.

The way in which hemoglobin absorbs light within the eye is obvious to anyone who has used an ophthalmoscope to examine an albino or a lightly pigmented person. The red pupillary reflex is another example of the optical importance of blood. The two forms of hemoglobin, oxygenated and reduced, have slightly different absorption curves, but both peak in the yellow portion of the spectrum. This property has been exploited by surgeons who seek to make burns in the retina, and some equipment manufacturers have taken up this argument to sell lasers that produce yellow light (e.g., certain dye systems). But it is not always realized that the curves that are produced in sales brochures showing how well hemoglobin absorbs in the yellow were made with path lengths many times longer than those found in retinal vessels or choroidal capillaries (Fig. 116-16). Path length is an important and, so it seems, often overlooked parameter, for A is related to it by Lambert's law, which, if expressed mathematically, may be rearranged thus:

$$A = 1 - e^{-bl} \qquad (10)$$

provided there is no loss due to scatter or reflection and β is the *extinction coefficient* for unit path length (constant at fixed wavelength) and l is the path length. If the path length is short, the value of A, and thus the amount of energy absorbed and the temperatures reached, will be reduced.

The value of the concept of penetration depth can now be put to good use. It requires no calculation to realize that if d for hemoglobin is 0.1 min in the yellow, to coagulate a blood vessel thermally it should not be much greater than about d in diameter or sufficient energy will not penetrate to its center. Unfortunately, if the vessel diameter is too small the bolus nature of blood flow may mean that the hemoglobin will not be equally distributed along the vessel and it is again equally difficult to coagulate the blood. It is possible to achieve blood coagulation by increasing the irradiance, but this is usually associated with destruction of tissues surrounding the target vessel, a situation that is not clinically desirable. It is, however, possible to produce hernostasis, but only if the target vessel is associated with some structure that is rich in melanin. The heat spreads from the melanin into the vessel to produce coagulation-of course, the melanin-containing tissues are also damaged.[11] Larger vessels are also difficult to close because the blood flows fast enough to carry away any

heat formed in the hemoglobin. Stopping the flow in target vessels by raising intraocular pressure improves the prospects for hemostasis, but it is easier to increase the irradiance, although this produces widespread damage beyond the vessel. The larger vessels usually run in the inner retina and are overlaid by the nerve fiber layer. When the vessels are exposed to intense radiation, the nerve fibers may be severed and their ends swell and form a diffuse, fluffy white mass called a cottonwoof spot that develops 12 hours or more after exposure.

Retinal thermal damage occurs only if the temperature of the target site rises, and it is easy to see that this cannot happen unless energy is delivered to the retinais actually absorbed in the tissue volume-faster than it leaks away. At the start of an exposure,

$$E_{ABS} = E_{LEAK} \qquad (11)$$

where E represents the energy being absorbed in, or leaking from, the tissue volume. Eventually an equilibrium may be reached where $E_{ABS} = E_{LEAK}$, and a steady temperature is established. At the end of the exposure, E_{ABS} is zero and the temperature decreases at a rate determined by E_{LEAK}. The time and temperature history of the tissue and its constituent molecules are generally held to be the parameters that determine how much damage is done, and this is expressed by the Arrhenius equation

$$\Omega = C_1 \int_{t_i}^{t_f} e^{-C_c/T(r,z)} dt \qquad (12)$$

where Ωis the fraction of molecules functionally destroyed for the temperature T between the initial time t_i to the final time t_f as a function of the radial distance r from the center of the retinal image along the z axis of the beam of incident radiation. Equations 11 and 12 could be the starting point of a model that could predict what exposure intensities and durations could lead to retinal damage.Indeed, many models have been based on this kind of argument, working back through the Arrhenius equation and considerations of E_{ABS} and E_{LEAK} to specify ocular hazards or the likely results of exposing eyes to a specified light source. Most authors have set Ωat 0.5 rather arbitrarily, it seems, if the arguments of Marshall[41] apply. The models encounter complexity, at least for n on-mathematicians, at the stage of calculating E_{ABS} and E_{LEAK} which values depend not only on the kinds of optical transmission problems discussed earlier but also on the distribution and physical dimensions of the chromophores that absorb the radiation. The models consider only melanin, and most treat it as though it were spread in a thin, uniform layer under the retina: infinitely thin, Vos[42]; in a 10 µm layer[43-47]; or 4 or 5 µm layers, depending on species modeled.[12] Most of these models included absorption in choroidal melanin. They have enjoyed considerable success with longer-duration exposures (greater than 5 µsec) but were perhaps wanting at very short exposure times.

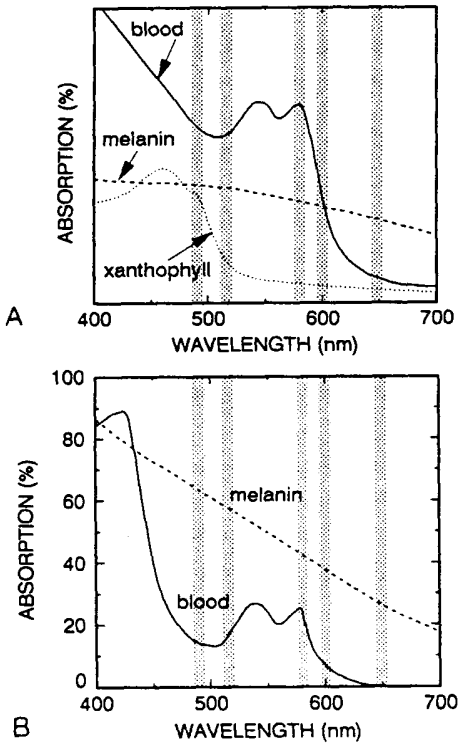

Figure 116-16. ***A*** Spectral absorption curve of blood (oxygenated hemoglobin?), melanin (solution?), and xanthophyll as they appeared in advertising material for ophthalmic laser instruments. Note, there is no scale on the ordinate axis, the source or nature of the curves is not specified, and no path lengths are given. ***B***, A version of ***A*** that seeks to rectify its errors. The melanin curve is taken from Gabel and coworkers[17](see curve *PG* in Figure 116-7) and represents data derived directly from RPE preparations. The blood curve is for a path length of 10 µm of oxygenated blood. Neither ***A*** nor ***B*** makes any allowance for transmission loss of preretinal media. Also, the five shaded bars are at the wavelengths of several common or new ophthalmic lasers: 488-nm argon, 514-nm argon, 580-nm dye, 600-nm dye, 647-nm krypton red.

Besides considering the likely damaging effects of temperature increases of several degrees producing tissue damage, Vos[42] proposed that steam production would be important for brief exposures. Eventually, it appeared necessary to redefine the models with the chromophore in granules rather than in a homogeneous slab, because of

difficulties with fitting the models to the data from short-exposure experiments. Hansen and Fine[48] proposed that heating of a granule of melanin would produce a zone of denaturation around it and that this would spread with conduction of heat away from the granule source; this is their single-mechanism model. They also proposed a two-mechanism model, in which steam would be generated at the granule-cytoplasm interface if the energy influx were great enough. The heating would lead first to thermal damage and then to steam and physical, disruptive damage. Hayes and Wolbarsht[49] also developed a granule model with which to explain the effects of steam generation in short exposures when power densities are usually high. They pointed out that there would be considerable amplification of lesion size (more than 1000 times) once steam was formed and that further amplification would come from the ensuing biologic effects of inflammatory processes.

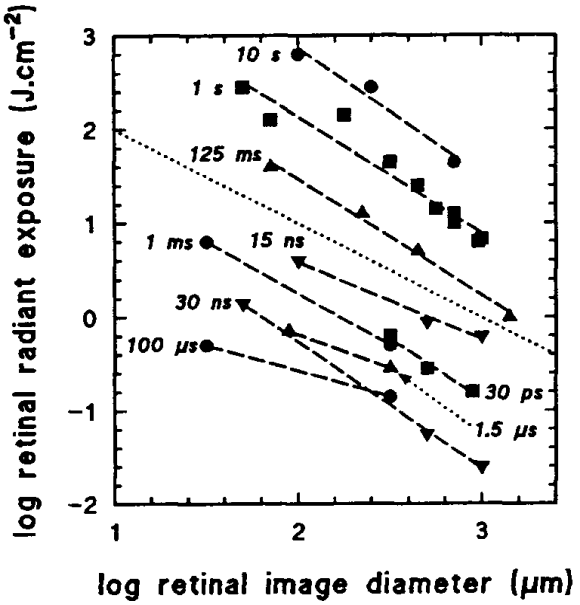

Figure 116-17. Log retinal radiant exposure for threshold lesions plotted against log of the diameter of the retinal image for various exposure times. The dashed lines are least squares fits through each set of data, and the dotted line has a slope of - 1. (Data from Sliney.[22])

Examination of the models is informative, and, indeed, they are still used today in certain circumstances, but a few simple considerations might help our understanding of thermal lesions in the retina without our becoming involved in calculus or numerical modeling. The way E_{ABS} may be defined is derived directly from Beer's and Lambert's laws and the considerations discussed earlier, but E_{LEAK} is more problematic.

Energy may leave the volume of the absorbing site by convection, not likely in a compartmentalized cellular structure, or by bulk flow of the chromophore, plainly the situation for hemoglobin in flowing blood in retinal vessels. Reradiation is not effective, as the temperatures are too low for Stefan's law to suggest other than infinitesimal flow. Conduction is the process by which most heat flows from the absorption site. The retinal image is usually circular and the chromophore is assumed to be in a homogeneously distributed layer, so the volume of the absorber increases with increasing retinal image diameter faster than the surface area of the absorber. As heat is lost through the surface, the relative rate of heat loss drops and larger images are associated with greater temperature increases at their centers or, put another way, the radiant exposure for threshold damage will fall. This is convincingly shown in Figure 116-17 for a range of exposure durations that have slopes approximating - 1. This is what simple surface area:volume ratios would suggest, as the ratio is inversely proportional to the radius of the image.

Exposure duration is a more important determinant of the type and extent of damage produced than is retinal image size. The concept of relaxation time[50] can help, because it defines the time that the temperature falls to 1/e of the initial value. It is related to the size of the absorbing chromophore by the relation

$$t_r = C \cdot l^2 \qquad (13)$$

where C is a constant (10^6s.m-2) and l is the smallest dimension of the heated object. For a typical melanin granule 1 μm in diameter, t_r is about 1 μsec and for an arteriole 100 μm, across, t_r is 10 msec. The smallest retinal images used in the laser clinic are typically 50 μm in diameter, which suggests values for t_r of about 2.5 msec. But the layer of melanin is only about 5 μm thick, which means t_r will be approximately 25 μsec. Consequently, if exposure times are greater than about this value, considerable heat will flow from the heated target chromophore to thermally damage the surrounding tissues. If laser surgical intervention is necessary in or near the fovea, prudence suggests that retinal image size should be kept small, but unless exposure time is also kept short there will be considerable heat flow, and perhaps greater vision loss than is intended.

The drive to shorter exposures should not be pushed too far, because, as the granule models of thermal damage explain and experiment shows, steam generation can become a problem with exposures of a few microseconds or less. Explosive generation of steam produces mechanical transient[51] that can tear tissues and even rupture blood vessels. If the exposure duration is very

short (e.g., a few nanoseconds from a Q-switched laser), the risk of hemorrhage is great. The ratio of the energy that just produces a hemorrhage to the energy for a just ophthalmoscopically visible retinal lesion falls from several hundred, with exposure durations of milliseconds, to near unity for exposures of a few nanoseconds (Fig. 116-18). This change can be interpreted to mean that, as the exposures get shorter the energy absorbed is transformed not only into heat but also into other modalities, such as mechanical forces.

The anomalies with the models of retinal light damage at short exposure times were referred to earlier. The models predict that there will be a relation between energy entering the eye, E_i, and exposure duration, t_e for threshold retinal lesions of the form

$$E_i = m \cdot t_e^n \qquad (14)$$

where m and n are constants and n is approximately 0.75. Fig. 116-19 shows this relation and some experimental data points. It is apparent that the relation holds for values of t_e> 20 μsec up to 10 sec, but that for exposure times less than that which corresponds to the thermal relaxation time of the RPE layer, the relation no longer holds. Below this thermal relaxation time, energy would not be expected to be lost from the absorbing chromophore and the energy input for threshold damage should be constant (i.e., the line in the figure should be horizontal). Obviously, the data points show a rise in threshold that peaks at durations of about 1 nsec. Sliney[22] suggests that this effect may be due to the conversion of the incident energy into mechanical energy (compression waves) or that the melanin acts as a saturable absorber, bleaching when the incident irradiance is too high and so letting some of the energy that would otherwise be expected to be absorbed pass through the target.

From the foregoing, it is clear that short exposures of around 100 nsec to 10 μsec are shorter than the thermal relaxation times of the RPE (but not of its individual granules) and thus on a gross scale will not cause heat flow into surrounding structures. Neither are these times short enough to lead to the mechanical problems that threaten exposures of a few nanoseconds or less. Consequently, they should be useful for limiting damage spread. If vital dyes or stains tied to immunohistochemically targeted cells or cell organelles could be administered to retinas, short-duration exposure to radiation matched to the absorption properties of the dye would allow selective destruction of these components.

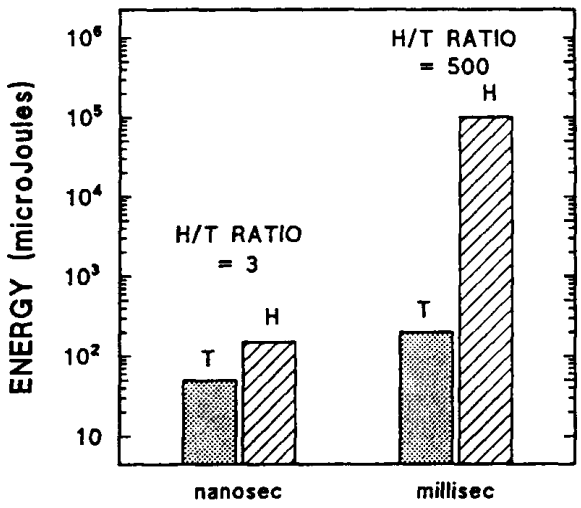

Figure 116-18. Ratio of the energy threshold for retinal hemorrhage to that required for a just visible thermal lesion (HIT) for two different pulse duration domains-nanoseconds (Q-switched laser) and milliseconds ("free-running" lasers). (Based on data from Wolbarsht and Allen[73] and other sources.)

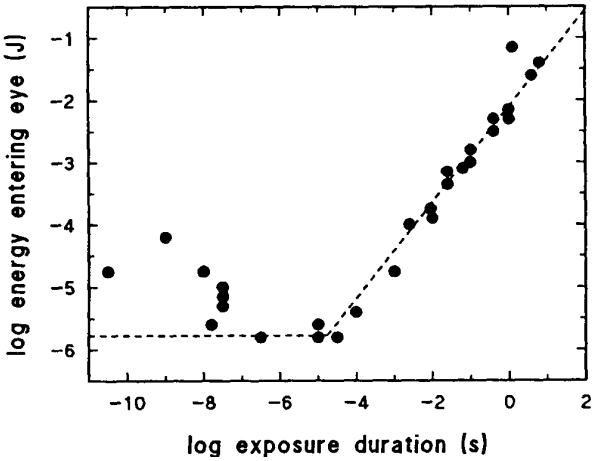

Figure 116-19. Log of the energy entering the eye that causes threshold thermal damage plotted against log exposure duration. The line has a slope of 0.75 and is a by-eye fit. (Data from Sliney.[22])

If the models describe long exposures well and very short exposures less well, there remains the problem of repeated exposure. Many light sources, lasers in particular, can be driven very effectively at high repetition rates; if the interpulse interval is less than the thermal relaxation time of the target chromophore there will be

accumulation of energy in the target, and more damage than expected will be produced. Put another way, the energy in each pulse of a train that just produces damage will be less than the threshold energy for a single pulse. Empirically it has been found that the energy content, Q_s, of a pulse in a train is reduced for threshold so

$$Q_s \propto N^{-0.25} \qquad (15)$$

where N is the number of pulses in the train. Sliney[22] describes how this relation applies over a wide range of pulse durations and repetition frequencies and to all but the largest image sizes. He argues that this is due to the interruption of a rapid repair process by the second and subsequent pulses. Examination of the times over which the relation holds suggests that the repair process must start within microseconds of the first exposure and be completed by about 1 second.

Figure 116-8 shows the spectrum of damage types, and for completeness thermoacoustic effects must be described. With sufficiently high retinal irradiance the explosive formation of steam and the generation of mechanical transients have already been mentioned. The importance of the mechanical transients lies in the way they trap some of the incident energy and carry it away from the site of the retinal image, perhaps over several millimeters. Similar but less intense mechanical energy can be generated without steam formation, owing to the rapid expansion of heated tissues that occurs with short (nanosecond-length) pulses.

By whichever mechanism the forces are generated (see under Photodisruption Damage), they promise damage of a different form to thermal denaturation and possibly at some distance for the retinal image. Marshall and Mellerio[52] showed that Q-switched laser pulses induced damage with mechanical displacement of cell constituents outside the image area, exactly as expected from the foregoing analysis. From their histologic examinations they were not able to say how much of the damage was due to the passage of the mechanical compression waves, the actual steam bubble surface, or the blood flow in hemorrhages that were often induced.[53]

Photodisruption Damage

As Figure 116-8 shows, when light exposure times are very short (nanoseconds and less), if enough energy is present to produce damage, this will be due to ionization and is usually called photodisruption. This ionization comes about because the strength of the electromagnetic field is so great that electrons are stripped from the outer orbitals of the atoms. Inside the eye this happens only when the radiation originates from Q-switched or mode-locked lasers and the beam is focused into a small volume either by accessory optical power of an ophthalmic instrument or contact lens or by the natural refracting power of the eye.

Photodisruption has found considerable use in recent years in ophthalmology, principally in the procedures of iridotomy to improve drainage of the posterior chamber and in posterior capsulotomy after implantation of an intraocular lens. In these and other procedures, the ionized tissue forms a plasma as the molecules are literally torn apart, thus enabling very small lesions to be made-an attribute that inspired the name "laser microsurgery". Further, it can be done inside the eye without opening the globe. Laser iridotomy and capsulotomy are both processes inflicted on anterior structures, but laser photodisruption has been used farther back in the eye. The problem of causing secondary damage to the retina when, for example, attempting to part a vitreal membrane, is very real and cannot be ignored.[54] Photodisruption might be considered a very good technique for removing epiretinal membranes because of the precise nature of the lesions formed, but there has been some concern that damage might be extended beyond the target site by mechanical processes (as described below[55]) or because the plasma is not generated exactly where expected but is displaced.` Because the incident beam has to be focused at the target site to ensure power density great enough to cause a plasma, the geometry of the beam in the focal area is an important determinant of whether and where a plasma is formed.[56] The cone angle of the focused beam specifies beam expansion beyond the focal region and thus determines the irradiance at the retina, which could be hazardous if the focus is too close to the retina.[57]

Taking values for retinal irradiance for threshold damage from the literature, Capon and coworkers[58] calculated how near the retina typical ophthalmic photodisruptor lasers can be focused before they become unsafe with a retinal irradiance that might cause a lesion

Table 116–3. MINIMUM FOCUS-RETINA DISTANCE (mm) FOR ND:YAG Q-SWITCHED LASER PULSES TO AVOID AN OPHTHALMOSCOPICALLY VISIBLE CHORIORETINAL LESION FOR THE LASER PULSE ENERGIES SHOWN

Beam Cone Angle (Degrees)	Energy (mJ)			
	6	12	20	40
32	2.8	3.9	5.1	7.2
24	3.8	5.3	6.9	9.7
16	5.7	8.0	10.4	14.7

Data from Reference 58.

(Table 116-3). The figures in Table 116-3 are probably optimistic, because they were derived for a minimal ophthalmoscopically visible retinal lesion, but fluorescence angiographic and electron microscopic techniques are more sensitive at tracing retinal damage.[59] If thresholds determined by these techniques are used, the minimum distances in Table 116-3 would probably need to be doubled. Consequently, a surgeon should use as high a cone angle as possible and, even with the minimum energy, must still avoid working within several millimeters of the retina unless he is prepared to accept the possibility of damage there.

The mechanisms of plasma formation are described elsewhere,[60] but they bear on the situation with the retina because the formation of a plasma in a liquid tends to occur at the site of an impurity or an interface.[56, 61] Consequently, if a Q-switched laser beam is brought to a focus in the vicinity of the retina, the inner surface of the retina may well be the site of plasma formation. Because the tissues at the plasma are vaporized the damage is considerable and the risk of hemorrhage very great. Even if the plasma is formed deeper in the retina or even in the choroid, because the focal volume of tissue is completely removed hemorrhage is very likely, and this could involve the vitreous (Fig. 116-20).[62] Visual function would be compromised, either by direct damage from the plasma to the neural elements or by the obscuring and delayed immunoreaction of the hemorrhage itself.

A plasma formed in a liquid environment, as in the eye, once initiated, loses heat (energy) very rapidly and is extinguished unless there is a sustaining inflow of energy. This is normally provided by the incident laser beam itself, so the plasma lasts only slightly longer than the laser pulse. Plasma maintenance absorbs energy from the laser radiation; in the early days of the development of photodisruption it was held that this absorption by the plasma would remove any hazard of radiation exposure to structures downstream from the plasma. This plasma shielding was investigated by several authors[63, 64] in conditions that approximate those in the eye, though over a range of energies that would be much higher than those normally used in the clinic.[65] The shielding was found to be variable and not to offer a very great safety factor at low-energy inputs of the level that are used in the clinic for iridotomy and capsulotomy.[58] It was estimates of the probable amount of plasma shielding that would arise in the attempt to section vitreal membranes that led to the figures shown in Table 116-3.

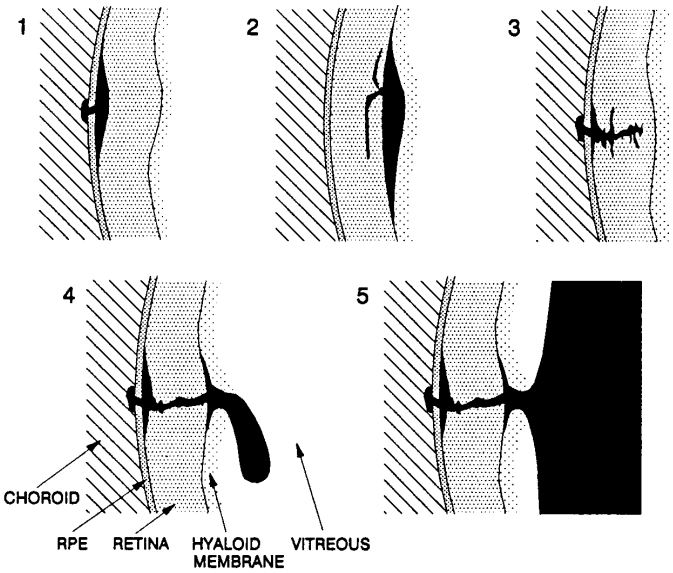

Figure 116-20. Diagram of different types of retinal hemorrhages that are produced by Q-switched or mode-locked laser pulses (blood if shown in solid black). 1, subretinal; 2, intraretinal and subhyaloid; 3, subretinal and intraretinal; 4, vitreous hemorrhage with a blood stalk trapped in the vitreous; 5, vitreous hemorrhage where the blood has mixed with the vitreous.

The generation of a laser-induced plasma by a Q--switched or a mode-locked laser leads to such extremely rapid expansion of the heated plasma[60] that a compression or shock wave is created that propagates away from the plasma throughout the eye. This wave is of concern because it carries energy to ocular locations remote from the initial site of exposure. The exact relationship between the magnitude of the shock wave and damage to tissues is one that still awaits elucidation, although it is well established in tissues other than the eye that there is a link between the two.[66-68] The bubble of heated gas left by the extinguished plasma expands for several milliseconds, and must damage surrounding tissues,[55] before collapsing destructively[69] or oscillating to release more energy in bubble pulses before the final destructive collapse. Should these cavitation bubbles be formed at or near the retina there is little doubt that considerable damage would be done by pressure waves and various shearing forces generated by jets and similar phenomena, in addition to the removal of tissue by the plasma itself. Indeed, it is hard to think of any therapeutic process that could be executed safely in or near the retina by photodisruptive methods because of the great risk of causing considerable tissue damage and massive

hemorrhage. Such exposure will probably be experienced only as a result of accident in industry or research, or of intent from battlefield laser weapons,[62] when the prognosis for full recovery of vision may not be good.

CONCLUSION

The study of the effects of light on the retina covers mechanisms that correspond to situations only slightly more intense than considered normal, up to frankly traumatic insults. Besides being fascinating, the study has practical implications, just as investigation of repetitive strain injury or of bone union in Colles' fracture has for a typist. Investigation of the processes that cause damage as a result of a lifetime's exposure to daylight, or to an industrial laser, have already borne fruit. Codes of practice for safe exposure to light sources now incorporate the knowledge derived from these investigations, and ophthalmic surgeons purposely harness damaging processes to manipulate tissue relationships. As our understanding of the way light interacts with tissues increases, we may look forward to better protection and more effective surgical intervention.

REFERENCES

1. Coohill TP: Action spectra revisited. J Photochem Photobiol [B] 13:95, 1992.
2. Sarna T: Properties and function of ocular melanin-a photobiological review. J Photochem Photobiol 12:215, 1992.
3. Mainster MA: Spectra, classification and rationale of ultraviolet protective intraocular lenses. Am J Ophthalmol 102:727. 1986.
4. Weale RA: Effects of the Ageing Lens on Vision. CIBA Sym posium No. 19. London, Elsevier, 1973.
5. Ijspeert J, Dewaard P, van der Berg T, et al: Intritocular stray light function in 129 healthy volunteers: Dependence on angle. age and pigmentation. Vision Res 30:699, 1990.
6. Weale RA: Human lenticular fluorescence and transmissivity and their effects on vision. Exp Eye Res 41:457, 1985.
7. Werner JS: Development of scotopic sensitivity and the absorption spectrum of human ocular media. J Opt Soc Am 72:247, 1982
8. Geeraets WJ, Berry ER: Ocular spectral characteristics as related to hazards from lasers and other light sources. Am J Ophthalmol 66:15, 1968.
9. Harwerth RG, Sperling HG: Prolonged color blindness induced by intense spectral lights in rhesus monkeys. Science 174:520, 1971.
10. Harwerth RG, Sperling HG: Effects of intense visible radiation on the increment threshold spectral sensitivity of the rhesus monkey. Vision Res 15:1193, 1975.
11. Fankhauser F, Kwasniewska S, van der Zypen E: Basic mechanisms underlying laser thrombogenesis in vascular structures of the eye. Lasers Light Ophthalmol 2:223. 1989.
12. Birngruber R, Hillenkamp F. Gabel VP: Theoretical investigations of laser thermal retinal injury. Health Phys 48:781. 1985.
13. Boulton M, Docchio F, Dayhaw-Barker P, et al: Age-related changes in the morphology, absorption and fluorescence of melanosomes and lipofuscin granules of the retina and pigment epithelium. Vision Res 30:1291, 1990.
14. Sarna T, SeAy RC: Photoinduced oxygen consumption in melanin systems: Action spectra and quantum yields for eumelanin and synthetic melanins. Photochem Photobiol 39:69, 1984.
15. Latimer P: Deconvolution of absorption spectra of green plant materials-improved corrections for the sieve effect. Photochem Photobiol 38:731, 1983.
16. Kollias N, Sayre RM, Zeise L, et al: Photoprotection by melanin. J Photochem Photobiol [B] 9:135, 1991.
17. Gabel VP, Birngruber R, Hillenkamp F: Visible and near-infrared light absorption in pigment epithelium and choroid. In International Congress Series **No. 450, XXIII, Concilium Ophthalmolo**gicum, Kyoto. Amsterdam, Elsevier, 1978.
18. Jukes T: Early development of the neutral theory. Perspect Biol Med 34:473, 1991.
19. Halliwell B, Gutteridge JM: Free Radicals in Biology and Medicine. Oxford, Oxford University Press, 1986.
20. Pooler JP, Valenzeno DP: Dye-sensitized photodynamic inactivation of cells. Med Phys 8:614, 1981.
21. Lingua RW, Parel JM: Photodynamic therapy for ocular tumors. J Photochem Photobiol [B) 9:119, 1991.
22. Sliney DH: Interaction mechanisms of laser radiation with ocular tissues. In Bennett HE, Guenther AE, Milan D, et al (eds): Laser-Induced Damage in Optical Materials. Washington, DC, NBS Publications, 1984.
23. Dillon J: Photophysics and photobiology of the eye. J Photochem Photobiol 1131 10:23, 1991.
24. Dayhaw-Barker P, Barker FM II: Phototoxicity: The problem of drug and chemical enhancement. In Cronly-Dillon JR, Marshall J (eds): Vision and Visual Dysfunction, Vol 16. London. Macmillan, 1991.
25. Marshall J, Mellerio J, Palmer DA: Damage to pigeon retinae by moderate illumination from fluorescent lamps. Exp Eye Res 14:164, 1972.
26. Ham WT, Mueller HA, Ruffolo JJ, et al: Basic mechanism underlying production of photochemical lesion in the mammalian retina. Curr Eye Res 3:165, 1984.
27. Mellerio J: Interaction of light with biological tissue and the potential for damage. In Cronly-Dillon JR, Marshall J (eds): Vision and Visual Dysfunction, Vol 16. London, Macmillan, 1991.
28. Noell WK, Walker VS, Kang BS, et al: Retinal damage by light in rats. Invest Ophthalmol Vis Sci 5:450, l966
29. Arden GB, Berninger T, Hogg CR, et al: A survey of colour discrimination in German ophthalmologists: Changes associated with the use of lasers and operating microscope. Ophthalmology 98:567, 1991.
30. Lawill T: Effects of prolonged exposure of rabbit retina to low intensity light. Invest Ophthalmol Vis Sci 12:45, 1973.
31. Rapp LM, Tolman BL, Dhindsa HS: Separate mechanisms for retinal damage by ultraviolet A and mid-visible light. Invest Ophthalmol Vis Sci 31:1186, 1990.
32. van Norren D, Schellekens P: Blue light hazard in rat. Vision Res 30:1517, 1990.
33. Ham WT, Ruffolo JJ, Mueller HA, et al: Histologic analysis of photochemical lesion produced in rhesus retina by short wavelength light. Invest Ophthalmol Vis Sci 17:1029, 1978.
34. Ham WT, Mueller HA, Sliney DH: Retinal sensitivity to damage from short wavelength light. Nature (Lond) 260:153, 1976.
35. Pautler EL, Morita M, Beezley D: Hemoproteins mediate blue light damage in the retinal pigment epithelium. Photochem Photobiol 51:599, 1990.
36. Kremers J.1M, van Norren D: Two classes of photochemical damage of the retina. Lasers Light Ophthalmol 2:41, 1988.
37. Kremers JJM, van Norren D: Retinal damage in macaque after white light exposure lasting 10 minutes to 12 hours. Invest Ophthalmol Vis Sci 30:1032, 1989.

38. Williams TP, Howell WL: Action spectrum of retinal light damage in albino rats. Invest Ophthalmol Vis Sci 24:285, 1983.
39. van Norren D: Photochemical damage to the eye. News Physiol Sci 6:232, 1991.
40. Sliney DH: New chromophores for ophthalmic laser surgery. Lasers Light Ophthalmol 2:53, 1988.
41. Marshall J: Lasers in ophthalmology: the basic principles. Eye 2 (Suppl):S98. 1988.
42. Vos JJ: A theory of retinal burns. Bull Math Biophys 24:115, 1962.
43. Clarke AM, Geeraets WJ, Ham WT: Equilibrium thermal model for retinal injury from optical sources. Appl Opt 8:1051, 1969.
44. Mainster MA, White TJ, Tips JH, et al: Transient thermal behaviour in biological systems. Bull Math Biophys 32:303, 1970.
45. Mainster MA, White U, Tips JH, et al: Retinal temperature increases produced by intense light sources. J Opt Soc Am 60:264, 1970.
46. White TJ, Mainster MA, Tipps JH, et al: Choroidal thermal behaviour. Bull Math Biophys 32:315, 1970.
47. Roulier A: Calculations of temperature increase in the eye produced by intense light. Bull Math Biophys 32:403, 1970.
48. Hansen WP, Fine S: Melanin granule models for pulsed laser induced retinal injury. Appi Opt 7:155, 1968.
49. Hayes JR, Wolbarsht ML: Thermal model for retinal damage induced by pulsed lasers. Aerospace Med 39:474, 1968.
50. Hillenkamp F: Laser radiation tissue interaction. Health Phys 56:613, 1989.
51. Mellerio J: Laser light and its effect on the retina. Trans Ophthalmol Soc UK 87:335, 1967.
52. Marshall J, Mellerio J: Pathological development of retinal laser photocoagulation. Exp Eye Res 6:303, 1967.
53. Marshall J, Mellerio J: Histology of retinal lesions produced with Q-switched lasers. Exp Eye Res 7:225, 1968.
54. Tassignon MJ, Stempels N, Brihaye M, et al: Vitreo-retinal membranes treated with the Q-switched Nd:YAG laser: A clinical study. Lasers Light Ophthalmol 1:205, 1987.
55. Mellerio J, Capon MRC, Docchio F: Nd:YAG lasers-a potential hazard for cavitation bubble behaviour. in anterior chamber procedures? Lasers Light Ophthalmol 1:185, 1987.
56. Capon MRC, Docchio F, Mellerio J: Investigation of multiple plasmas from ophthalmic Nd:YAG lasers. Lasers Light Ophtbalmol 1:147, 1987.
57. Bonner RF, Meyers SM, Gaasteriand DE: Thresholds for retinal damage associated with the use of high power Nd:YAG lasers in the vitreous. Am J Ophthalmol 96:153, 1983.
58. Capon MRC, Docchio F, Mellerio J: Nd:YAG laser photodisruption: An experimental investigation on shielding and multiple plasma formation. Graefes Arch Clin Exp Ophthalmol 226:362, 1988.
59. Boriand RG, Brennen DH, Marshall J. et al: Role of fluorescein angiography in the detection of laser-induced damage to the retina: A threshold study for Q-switched neodymium and ruby lasers. Exp Eye Res 27:471, 1978.
60. Docchio F, Regondi P, Capon MRC, Mellerio J: Study of the temporal and spatial dynamics of plasmas induced in liquids by nanosecond Nd:YAG laser pulses: 1. Analysis of the plasma starting times. Appl Opt 27:3661, 1988.
61. Docchio F, Dossi L, Sacchi CA: 0-switched Nd:YAG irradiation of the eye and related phenomena: 1. Optical breakdown determination for liquids and membranes. Lasers Life Sci 1:87, 1986.
62. Mellerio J. Marshall J, Tengroth B, et al: Battlefield laser weapons: an assessment of systems, hazards, injuries and ophthalmic resources required for treatment. Lasers Light Ophthalmol 4:41, 1991.
63. Loertscher H: Laser induced breakdown for ophthalmic applications. In Trokel SL (ed): YAG Laser Ophthalmic Microsurgery. Norwalk, CT, Appleton-Century-Crofts, 1983.
64. Steinert RF, Puliafito CA, Kittrel C: Plasma formation and shielding by three ophthalmic Nd:YAG lasers. Am J Ophthalmot 96:427, 1983.
65. Steinert RF, Puliafito CA, Kittrel C: Plasma shielding by Q-switched and mode locked Nd:YAG lasers. Ophthalmology 90:1003, 1983.
66. Ara G. Anderson RR, Mandel KG, et al: Irradiation of pigmented melanoma cells with high intensity pulsed radiation generates acoustic waves and kills cells. Lasers Surg Med 10:52, 1990.
67. Suneson A, Hansson HA, Lycke E, et al: Pressure wave injuries to rat dorsal root ganglion cells in cultures by high energy missiles. J Trauma 29:10, 1989.
68. Yashima Y, McAuliffe DJ, Flotte TJ: Cell sensitivity to laser induced photoacoustic injury of the skin. Lasers Surg Med 10:280, 1990.
69. Vogel A, Lauterborn W, Timms R: Optical and acoustical investigation of the dynamics of laser-produced cavitations near a solid boundary. J Fluid Mech 206: 299, 1989.
70. Sliney DH, Wolbarsht M: Safety with lasers and other optical sources. New York, Plenum Press, 1980.
71. Maher EF: Transmission and Absorption Coefficients for Ocular Media of the rhesus Monkey. Report SAM-TR-78-32, USAF School of Aerospace Medicine. Brooks Air Force Base, Texas, 1978.
72. Mellerio J: Yellowing of the lens-nuclear and cortical contributions. Vision Res 27:1581, 1987.
73. Wolbarsht M, Alien R: New laser device for ophthalmology: A comment. Appl Opt 25:1533, 1986.