Retinal damage by light in rats

Werner K. Noell, Virgil S. Walker, Bok Soon Kang,* and Steven Berman

The retina of laboratory rats is affected irreversibly by intense light applied for less than 1 hour or for up to 2 days depending upon experimental conditions. Exposure of unanesthetized and unrestrained animals was in chambers surrounded by a green filter and circular fluorescent lamps of a nominal brightness of 2,040 footlamberts. Eyes of anesthetized animals were exposed diffusely to either the light from a 100-w. zirconium arc passing through filters or monochromatic light of various wavelengths. Irreversible reduction in ERG amplitudes and degeneration of visual cells and pigment epithelium indicated the severity of the light damage. The effect was very dependent upon the body (eye) temperature during exposure. Hyperthermia greatly accelerated and intensified the damaging action of light and for this reason most experiments reported in this paper were performed at a high body temperature. At a body temperature around 104° F. severe damage was produced with exposures to 5 to 10 μw per square centimeter retina for 1 hour. The minimal damaging dose at a high temperature was estimated to be about 1 μw per square centimeter. The action spectrum of the damaging effect approximated that of visual excitation as measured by the ERG. Hooded (pigmented) animals were no more affected than albinos of different strains. Recovery in the dark from a just subliminally damaging dose of light at a high body temperature required about 24 hours and was preceded by a period of time during which the retina was "sensitized" to an additional dose. During or following exposure to light at a high body temperature visual cell and pigment epithelial damage developed about simultaneously and was first indicated by pyknosis and cell swelling followed rapidly by the dissolution of nuclei and cytoplasm. The crucial reaction in producing the damage is considered a "dark-reaction" initiated by light of an intensity which bleaches measurably rhodopsin. Hypotheses on the reaction sequence which leads to damage are briefly discussed.

In the following a surprising effect of light upon the retina of albino and pigmented rats will be described. It was discovered when "normal" unanesthetized and unrestrained rats were maintained continuously for 24 hours in an environment illuminated by ordinary fluorescent light bulbs.¹ The electrophysiological and histological manifestations of this effect will be presented, as well as studies performed to evaluate the variables involved.

The interest in this effect rests mainly on the assumption that any unusual vulnerability of the retina to physical or chemical agents may relate to the cellular abnormalities which lead to retinal degeneration on a hereditary basis.

Methods

The experiments to be reported were performed on 590 rats each exposed to light of various kinds and for varying periods of time. The animals ranged in weight between 100 and 400 grams, but most were approximately 180 grams at the time they were exposed to light. They were from

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inbred stocks and purchased mainly from the Charles River Farm. Unless differently specified, the experiments were performed on the CDC strain of this source which is a strain of albinos.

The animals were purchased generally two weeks prior to use and kept after arrival in artificially and weakly illuminated quarters, regulated to have a temperature of 24° C. Prior to exposure they spent at least 24 hours in a dark room illuminated only by dim red light for short periods of time.

For exposing the animals to light, essentially three kinds of procedures were used. In Procedure No. 1, the unanesthetized, unrestrained animals were placed in an ordinary circular wire mesh cage or a glass desiccator surrounded by a curved sheet of transparent, acrylic plastic of % inch thickness. Unless differently specified in Results, the plastic was green in color and transmitted through the range from 490 to 580 mÅ (Fleeglas No. 2092). Around the plastic were fitted vertically 3 circular 32 w. fluorescent lamps (12 inches in outer diameter) of the General Electric Circline, Cool White type. The nominal brightness of these lamps is 2,040 footlamberts.

On occasion, different types of light were used in conjunction with different filters. The 3 lamps provided directly by these lamps through the filter was excluded from reaching the chamber. Light other than that than 2 inches higher or lower than the area covered by the lamps. Light other than that provided directly by these lamps through the filter was excluded from reaching the chamber by a light shielding cylinder on top of the chamber. Measured illumination within the chamber ranged from 1,200 to 2,500 lux depending upon the distance from the chamber wall.

The chambers were adequately ventilated by a wide stream of air so as to keep measured CO₂ concentration normal. In addition CO₂ accumulation was prevented by soda lime whenever the experimental arrangement imposed limitation on the ventilation of the chamber. Oxygen pressure within the chamber was varied between 60 and 700 mm. Hg in one series of experiments. In the principally used design, 4 of these chambers with their lighting individually controlled, were separately housed within a large box in a temperature-controlled room. The temperature and humidity of the chambers were continuously monitored and could be adjusted as desired. On occasion, the assembly was kept in an "all-weather" room in order to test special temperature conditions.

Rectal temperature was measured prior to and after light exposure and at intervals of 1 to 2 hours during exposure. Each cage was equipped with food and water. During prolonged exposure to a high temperature the animal could choose between ordinary water and a saline solution.

Two to four animals were kept within one cage so that as many as 16 animals could be experimented with at the same time.

In Procedure No. 2, the animal was exposed to monochromatic light through a Grating Monochromator* equipped with a 200 w. direct current xenon-mercury compact arc lamp. Radiation from a spectral region coinciding with a mercury line either was focused upon the eye or illuminated a sheet of translucent plastic. In the latter case, the smallest diameter of the illuminated area was 4.5 cm.

The animals were anesthetized with pentobarbital Sodium to a depth which occluded eye movements. The right eye of each animal was placed 1 cm. behind the illuminated disc and kept open by black adhesive tape. The pupil was maximally dilated by atropine. During light exposure the animal was in a box partitioned in such a way that the air temperature around the eye and the hind part of the body could be adjusted separately in order to keep the air temperature and humidity at the eye constant while the temperature was changed over a major heat exchanging part of the body for the maintenance of body and blood temperature at the desired level. The constancy of rectal temperature depended upon a constant depth of anesthesia which was controlled by repeated injections of the anesthetic in small doses through an intraperitoneal cannula; decreasing depth of anesthesia generally was first signalled by a fall of the rectal temperature when environmental temperature was high. Direct control of body temperature was achieved by a variable flow of air of the desired temperature and humidity through the chambers. Prior to exposure the animals were brought in the dark to the desired body temperature and maintained at this level for about 1/2 to 1 hour until exposure to light was started. This was the routine also for Procedure No. 1.

Ocular temperatures were not measured in these experiments but control studies indicated that the retinal temperature of these eyes is a function of the temperature of the blood and of the ocular environment. For these reasons the temperature gradient between blood and ocular environment was kept low and air humidity high whenever the experiment demanded the utmost constancy of conditions. For instance, when, for the determination of the action spectrum of the light effect, rectal temperature was maintained near 104° F. (40° C.), air temperature around the eye was kept at 39° C. and humidity above 70 per cent. In order to avoid confusion, environmental temperature will be given in centigrades and rectal temperatures in degrees Fahrenheit (° F.).

Procedure No. 3 was the same as No. 2 except *Bausch & Lomb, No. 33-85-45.
*Hanovia.
that the light source was a 100 w. zirconium arc illuminating the opal plastic over a circular field of 7.5 cm. diameter, 1 cm. in front of the rat's eye. The light passed through a liquid copper sulfate filter of measured spectral transmittance and through a calibrated No. 64 Kodak-Wratten filter with peak transmission at 500 mμ. Maximal luminance of the disc was about 1,000 equivalent lux.

Photometric measurements were made with a calibrated Weston meter, Model 603. A calibrated thermopile (Yellow Spring Model 65) served radiometric measurements. Calibrated neutral density filters were used to vary light intensity. Light sources were continuously monitored by photocells and electronic means. In addition to the physical measurements, the radiations used were measured by means of their effectiveness in eliciting the ERG of a dark-adapted control animal. For these measurements, radiation was applied as a flash of 100 msec. duration while the anesthetized animal was in the same position as during prolonged exposure except that its body temperature was maintained at the physiological level. Light intensities were varied over a wide range in order to make threshold determinations. The electrical responses were recorded on film from a cathode ray oscilloscope.

The state of retinal functioning following exposure to light was determined by means of a standardized ERG test procedure during which the animal was under pentobarbital anesthesia. Both eyes were tested in sequence and the sequence repeated once. The stimulus was a 10 μsec xenon arc flash (Grass photic stimulator) applied at a rate of 1 every 2 to 5 minutes when the stimulus was more than 4 log units above b-wave threshold. The amplitudes of a- and b-waves in response to the strongest stimulus (scale reading 16) were used as the basis of comparison.

Following prolonged exposure to light (Procedures 1, 2, and 3) the animal was continuously kept in the dark room. Routinely, the first ERG test was performed 24 hours after exposure; several other tests followed at intervals of 2 to 7 days. Control animals (in darkness for at least 24 hours) were tested separately and in conjunction with the testing of the affected animals. The experimental animals were killed after about 3 weeks and their eyes fixed in Zenker solution. Slides from approximately 200 eyes were examined.

Results

Manifestations of the damaging effect of light. Fig. 1 shows the ERGs of 3 groups of a total of 9 animals recorded during the routine test in response to a maximal stimulus (10 μsec xenon arc flash). The test was performed about one week after exposure to light in accordance with Procedure 1. The group of animals from which the ERGs of the right-hand column (dark) of Fig. 1 were recorded serves as control. These animals were kept for 24 hours in an exposure chamber of the same design as used for the other groups except that the chamber was shielded by dark material from the light of the fluorescent bulbs surrounding the chambers. The ERGs of these animals are well within the range of the normal variations observed in a naive control sample. The ERGs of the left-hand column are from 3 animals which in the same kind of chamber were exposed to the fluorescent light through the green plastic filter (No. 2092, see Methods). Exposure time was 24 hours as in all other animals of this figure. Evidently, the ERG (a- and b-wave) of each animal is reduced in amplitude compared to the control series. The ERG of the other eye was also always tested and, generally, when the ERG of one eye was reduced that of the other eye was reduced as well and to about the same degree. For instance, when exposure (Procedure No. 1) had conditioned an ERG reduction of about 75 per
cent, the mean difference between the two eyes of the same animal was less than 10 per cent of the control amplitude. In contrast, when only one eye was exposed to light (Procedures Nos. 2 and 3) and irreversibly damaged, the ERG of the other eye was within the normal range.

It is evident (Fig. 1) that the reduction in ERG amplitude varies among animals. These variations, however, are not the result of a variable related to the ERG test procedure. With rare exceptions, the degree of ERG reduction in any animal was persistently about the same with repeated testing over a period of 3 to 4 weeks provided the first test followed exposure after at least 24 hours. The variations in response, therefore, relate to variations in the retinal effectiveness of light.

Because ERG reduction was persistent, the effect of the 24 hour exposure to light appeared to be an irreversible one which from experience with other agents was expected to be the result of irreparable tissue damage. This was confirmed by histologic examination. Whenever the ERG of an eye was for several weeks reduced by 30 per cent or more, a histologic section through the equator of the eye revealed at least a small retinal area over which the visual cell layers were either completely destroyed or reduced due to the destruction of a fraction of the population.

During the initial part of this study all animals seemed to be affected irreversibly when exposed for 24 hours according to Procedure No. 1, but variations among experiments were very large until environmental and body temperature were controlled and maintained at a steady level. The damaging effect of light varied with body (and eye) temperature during the exposure and was more pronounced. This was shown in Fig. 1 by the ERGs of the 3 animals of the middle column. Whereas in the experiment of the left-hand column the temperature of the chamber was maintained near 28.5° C, the animals of the middle column were exposed to the light at an environmental temperature of around 36° C. Clearly, these animals are affected to a much greater degree than the others. The histological damage of their retinas also was more extensive. In contrast, maintenance of an animal at a high temperature in the dark for as long as compatible with the animal’s survival proved to be ineffective, i.e., no irreversible effect was produced. On the other hand, every animal out of a group of 62 showed more than 50 per cent irreversible ERG loss when exposed at a temperature of 39° C. to the light of Procedure No. 1 for only 2 to 4 hours. All variables related to the damaging effect of light, therefore, need to be discussed in relation to temperature during exposure. Because this damage by light was produced much faster at an elevated body temperature than at the normal temperature, the experiments of this study (i.e., the exposures to light) were generally performed during hyperthermia.

The damaging effect of light will be expressed in the following as a quantity in terms of the irreversible ERG loss, specifically by the reduction in the a-wave amplitude which proved to be a more consistent index than that of the b-wave. Unless specified differently, the ERG 24 hours after exposure will be used for the comparison of different conditions.

Age above 50 days and sex did not seem to influence the results; however, the comparison of different conditions was always made with animals of the same sex and weight. The damaging effect also was not significantly altered by pentobarbital anesthesia during exposure. Similarly no differences in the effectiveness of light were noted when the animal’s food was from different commercial sources or specially prepared.

Relationship of the irreversible effect to exposure time and body temperature. The results obtained with varying exposure time at 3 different levels of body temperature was summarized in Tables I to III. Control amplitude for the a-wave was on the average 660 μv plus or minus a stan-
Table I

<table>
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<tr>
<th>Exposure time (hours)</th>
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<th>b-wave μV ± SD</th>
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<tr>
<td>600 ± 70</td>
<td>540 ± 230</td>
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<tr>
<td>1,260 ± 190</td>
<td>1,020 ± 370</td>
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Table II

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<td>Control</td>
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<th>a-wave μV</th>
<th>b-wave μV</th>
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<tr>
<td>660 ± 70</td>
<td>585 ± 135</td>
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<tr>
<td>500 ± 90</td>
<td>445 ± 190</td>
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<td>230 ± 160</td>
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<td>70 ± 60</td>
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<td>1,260 ± 190</td>
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<td>530 ± 300</td>
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Table III

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<th>b-wave μV</th>
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<td>660 ± 70</td>
<td>360 ± 110</td>
</tr>
<tr>
<td>240 ± 60</td>
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<td>130 ± 90</td>
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<tr>
<td>460 ± 320</td>
<td>410 ± 370</td>
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<tr>
<td>225 ± 270</td>
<td>90 ± 110</td>
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<tr>
<td>1,220 ± 240</td>
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Standard deviation of 70 μV derived from a group of 20 animals (40 eyes) tested at different times during the study. The average b-wave amplitude of these animals was 1,260 μV ± 190. Table I lists the results of the ERG test performed on 43 animals 24 hours after they had been exposed to light within the chambers of Procedure No. 1 (see Methods) while environmental temperature was 34.5° C. and exposure time 2 to 12 hours. These animals comprise a group for which average rectal temperature during exposure was within the limits of 99.8° and 100.9° F. (average around 100.4° F.). A statistically significant reduction in ERG amplitude (denoting irreversible ERG loss as indicated by repeated testing) was apparent for exposure times equal to or exceeding 6 hours. Continued maintenance of the animals for 12 hours in the light environment resulted in an average reduction of the ERG to ap-
proximately one-third the control amplitudes. Longer exposure led to a more severe effect as indicated in Table I (last column) by a group of animals with an average body temperature of 99° F. during a 20 hour exposure at an environmental temperature of 26° C.

It is clear from Table I (last column) that damage by light is apt to occur even when body and environmental temperatures are within the normal range. Severe effects were observed also when rectal temperature was as low as 97.5° F. and ambient temperature 15 to 20° C., but in order to produce the effect at this temperature level continued exposure for 48 hours was required.

The level of retinal susceptibility to light was apparently set by the body temperature. The rectal temperature of the animals listed in Table II was between 102.3 and 103.2° F. As shown, a highly significant reduction of the ERG was produced with only 1 hour of exposure to the same light as in Table I. Apparently, the higher the body temperature above normal the shorter the time required for the light of a given intensity to produce an effect.

Table III lists the results obtained when environmental temperature was close to 39.5° C. and rectal temperature as high as 104° F. Surprisingly, significant effects developed when exposure was as short as 10 minutes. Exposure of 4 hours to the light at this temperature resulted in practically the complete irreversible loss of the ERG. In contrast, exposure to heating alone (Table III, last column) was ineffective as was illustrated also in Fig. 1.

Fig. 2 illustrates in graphic form the relationship between the effect of light and body temperature. It indicates that with a constant light intensity and a constant exposure time of 4 hours the range from minimal to almost maximal effect relates to a body temperature extending from 100 to 102.5° F. (or 37.5 to 39° C.). The capacity of light to produce damage hence appears to be extremely dependent upon the tissue temperature within an amazingly narrow range.

The second important variable for producing damage, exposure time, is graphically illustrated in Fig. 3, light intensity and body temperature (100° F.) being constant while exposure time varies. As shown, constant light has a cumulative action. At a temperature of around 100° F., the range from minimal to almost maximal effect appears to correspond to a

Fig. 2. Relationship between the ERG in per cent of control amplitude 24 hours after light exposure and the body temperature during exposure. Exposure was 4 hours for all line-connected points. Numbers above each point denote the number of animals used; each point represents the average ERG amplitude of 4 to 14 animals.
Fig. 3. Relationship between ERG 24 hours after exposure and the duration of exposure. Exposures for 2 to 12 hours were performed at a rectal temperature during exposure varying on the average between 100.2 and 100.5° F; the 20 hour value is from a group of animals with a body temperature near 99° F. Standard deviations are indicated for the a-wave only.

time span extending from a relative value of 1 to one of about 10 (e.g., from 2 to 20 hours). Approximately the same relationship seemed to hold for high body temperature at which the absolute time scale was considerably shortened.

The third variable, light intensity, will be discussed in a subsequent section (effective retinal illumination), where it will be shown that minimal effects become almost maximal ones with a change in light intensity of less than 1.5 log units. Using a large number of animals, it would be possible to interrelate these three variables and to predict the damage for a given set of conditions. We were prevented from doing so by the relatively great variations within the range from minimal to maximal damage as indicated by the standard deviations (see Tables I to III, Fig. 3). For the unanesthetized, unrestrained animals (Procedure No. 1) these variations could be explained by the lack of control of actual light exposure but they were evident also when one eye was steadily exposed in accordance with Procedures Nos. 2 and 3. It is our impression, therefore, that there is at least one parameter of the effect which so far has remained uncontrolled despite efforts to find it by varying conditions, e.g., the dark period preceding exposure and the duration and levels of pre- and post-exposure hyperthermia. None of these were found to be very important.

Recovery from exposure to light. In previous work on the effects of x-irradiation upon the retina various dose schedules of exposure were tested in order to obtain evidence on the rate of recovery from any reversible "damage" produced. In a similar way, the irradiation by light was interrupted for varying periods of time during which the animal was in darkness. A cumulative action of light became apparent even when the interval between exposures measured several hours. Illustrative examples of this are given by the data listed in Tables IV and V, which comprise only a small part of the experiments performed on this point.

The experiments of Table IV demonstrate the cumulative action of a 5 minute exposure (Procedure No. 1) at an environmental temperature of 36° C. and a rectal temperature around 103° F.
Table IV

<table>
<thead>
<tr>
<th>No. of doses*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>1†</th>
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<tbody>
<tr>
<td>Total exposure time (min.)</td>
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<td>10</td>
<td>15</td>
<td>20</td>
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<tr>
<td>a-wave</td>
<td>Control 660 ± 70</td>
<td>580 ± 125</td>
<td>580 ± 130</td>
<td>370 ± 150</td>
<td>180 ± 90</td>
</tr>
<tr>
<td>b-wave</td>
<td>1,260 ± 190</td>
<td>1,150 ± 160</td>
<td>1,040 ± 260</td>
<td>710 ± 250</td>
<td>450 ± 200</td>
</tr>
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</table>

*One-hour interval between exposures (doses).
†Continuous exposure for 20 minutes.
†ERG test 24 hours after last exposure.

Table V

<table>
<thead>
<tr>
<th>No. doses applied</th>
<th>Total exposure time 1 hour</th>
<th>4–6</th>
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<tbody>
<tr>
<td>Interval between doses</td>
<td>Continuous</td>
<td>6 hours</td>
</tr>
<tr>
<td>a-wave*</td>
<td>Control 600 ± 70</td>
<td>310 ± 100</td>
</tr>
<tr>
<td>b-wave*</td>
<td>1,360 ± 150</td>
<td>780 ± 205</td>
</tr>
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</table>

*ERG test 5 days after last exposure.

maintained constant for a total of 5 hours. Applied singly at different times during the period of hyperthermia, exposure for 5 minutes did not produce a significant effect. The same was true for 2 exposures separated by a one hour interval. However, 3 and 4 exposures, each of 5 minutes' duration and each following the preceding one by a one hour dark interval, led to significant damage. Obviously, the interval of one hour was not sufficiently long for recovery from the changes induced by the illumination. Even more surprising was the finding that dose fractionation was apt to produce a more severe effect than when illumination was for the same total duration without interruptions. This is shown by the last two columns of Table IV where the effect of a continuous exposure for 20 minutes can be compared with that of 4 doses of 5 minutes' duration each. The latter effect is significantly more severe.

In the experiments of Table V total exposure time was one hour while the chambers (same conditions as in Table IV) were illuminated either continuously or for periods of 10 or 15 minutes with dark intervals of 6, 24, or 48 hours. (Results with the 10 and 15 minutes' exposures were very similar and the data obtained were lumped.) Body temperature was permitted to fall to normal during the dark interval after having been elevated for a period of about two hours during which the exposure to light was performed. When the interval between exposures was 6 hours, the effect was more severe than for the 1 hour continuous illumination which resulted in almost 50 per cent irreversible loss in ERG amplitude. However, when the interval was 24 or 48 hours, no effect was obtained.

It is concluded (a) that the changes induced by a brief and intense illumination probably have not subsided much earlier than 24 hours after an exposure which is just subliminal for producing irreversible damage (cf. Table IV) and (b) that recovery from such an exposure is preceded by a period of time during which the retina is sensitized to the damaging effect of additional illumination.

Theoretically, ERG testing beginning
immediately after exposure to light could reveal the rate of recovery from reversible damage provided this recovery is (or has become) the rate-limiting process of dark adaptation. In view of the normally slow dark adaptation of the rat retina this was not expected to yield easily analyzable data and only a qualitative description is possible at this time. Procedures Nos. 1 and 3 were utilized in these experiments. With Procedure No. 3, the ERG of the exposed eye was recorded during and immediately after exposure to light of 200 equivalent lux disc luminance in response to a strong xenon-arc flash applied every 5 or 10 minutes. Utilizing Procedure No. 1, the ERG was measured as in the routine test VA, V2, 1, 3, 6, and 24 hours after a standard exposure which varied in duration from 5 minutes to 4 hours. Body temperature during all exposures was elevated to about 106° F.

Except when the ERG had been completely abolished during exposure, the cessation of light was followed by an immediate rise in ERG amplitude, the slope of this rise inversely related to the duration of exposure and the magnitude of the irreversible ERG loss. When exposure (Procedure No. 1) was such as to produce an irreversible effect, ERG recovery proceeded for about 3 hours to be followed by a slow decline so that a test 24 hours after exposure showed an ERG which in amplitude was only a fraction of that recorded after 3 or 6 hours (Figs. 4A and 4B).

Superficially viewed this would indicate that retinal responsiveness after a brief exposure is the resultant of two simultaneous processes each probably being complex, a process of recovery from changes induced by light and a process of progressive deterioration in cell function which extends far into the post exposure period.

Analytical separation of these two processes is made difficult by the fact that an impairment of retinal function may be initiated by an increase in ERG amplitudes. This has been observed during x-irradiations.

Fig. 4A. ERG of the left and right eyes of a rat before and after exposure (1 hour to 7 days) to the light of Procedure 1. Exposure was for one hour at a chamber temperature of 39° C. (rectal temperature 103.6° F. on the average during exposure). The animal was unanesthetized during exposure but under pentobarbital anesthesia during the ERG test. Following exposure the animal was kept in the dark; each ERG test entailed exposure to 2 flashes of light per eye. The ERG 7 days after exposure was the same as recorded 2 weeks later.

Fig. 4B. Similar experiment to that in A, except that exposure time for both animals (Nos. 11 and 12) was 30 minutes. The ERGs were recorded between 3 hours and 7 days after exposure. At 21 days after exposure, ERG amplitudes were within plus or minus 15 per cent of the 7 day records.
tion and also during exposure to a high oxygen pressure. The initial increase in ERG amplitudes following exposure, therefore, may be an exaggerated manifestation of recovery from light because the ERG amplitudes are abnormally high. This interpretation is probably correct because the ERG tested during exposure (Procedure No. 3) in response to a superimposed flash of light also showed an increase beginning about 45 minutes after light-on (200 equivalent lux) and reaching within one hour an amplitude value which was up to twice as high as that measured earlier during exposure. Following this increase, the ERG slowly and steadily decreased while exposure continued.

Despite these complex considerations, the experiments just described clearly indicate that the damaging reaction initiated by light progresses slowly even in subsequent darkness and even while retinal excitability undergoes a similar change as during physiological dark adaptation.

In the majority of our experiments, ERGs which 24 hours after exposure were reduced by 20 per cent or more from control, tended to become lower during the next seven days but thereafter remained at a steady level. Generally the decrease after 24 hours amounted to no more than 10 per cent of the control amplitude. In a minority of experiments the ERG indicated recovery during the period following 24 hours; on occasion, such a recovery was observed to occur during the second week after exposure. In these cases, the ERG recorded 24 hours after exposure was reduced by less than 50 per cent. All these data relate to experiments in which exposure to light was performed during hyperthermia.

**Effective retinal illumination.** In order to evaluate the physical variables of light the animals were under anesthesia and exposed to Procedures Nos. 2 and 3. Average results relating the irreversible damage (ERG tested 2 to 5 days after exposure) to the intensity of the light are plotted in Fig. 5. Light intensities are given on a log scale in multiples of the threshold intensity for eliciting the a-wave determined by presenting the light as a 100 msec, stimulus in a dark-adapted, control animal. The lights used in these experiments were the green filtered zirconium arc light and

![Fig. 5](image-url)

**Fig. 5.** Relationship between the ERG several days after exposure and the intensity of retinal illumination expressed in multiples of a-wave threshold. Exposure was either to zirconium light through a green filter or to the mercury line at 5,461 A, both lights illuminating an opal plastic in front of the eye. The animals were anesthetized during exposure (1 or 3 to 4 hours); rectal temperature during exposure was in each experiment above 104° F. At 2.5 log units above a-wave threshold, the zirconium light incident upon the eye was 11 μW per square centimeter and that of the 5,641 A radiation 6 μW per square centimeter (see text).
monochromatic radiation of 5,461 A, both illuminating a large area of the opal plastic in front of the eye (see Methods).

Fig. 5 shows that for a given time of exposure the range from minimal to maximal effect extended over a range of no more than 1.5 log units light intensity. With a one hour exposure at 104° F., the threshold of effectiveness was about 2 log units above the a-wave threshold while for a 3 to 4 hour exposure it seemed to have been lower than that by about 0.5 log unit. At an intensity of 2.5 log units above a-wave threshold, the irreversible ERG loss was about 30 per cent following a one hour exposure and about 100 per cent when exposure time was 3 hours. For relating these effects to b-wave threshold about 4 log units would have to be added, this difference between a- and b-wave threshold being consistent with many other data of our laboratory and with the measurements reported by Dodt and Echte.

In carefully conducted experiments, Cone related the a- and b-wave amplitudes of the albino rat to the illumination of a 110 degree retinal field in terms of the average number of quanta absorbed per rod. His data indicate that a 20 msec. stimulus (5,000 A radiation) 2.5 log units above the a-wave threshold corresponds to an average of 1,000 quanta absorbed per rod, taking 0.5 log intensity on his scale as the a-wave threshold. Converting this number of quanta to radiant flux per unit area through the surface of the retina gives a value of 3.5 x 10^-6 joules per second per square centimeter, using his measurements for the retinal area of 110 degree field (0.17 cm.²), the rod number per square millimeter (40 x 10⁴), and the ratio of quanta absorbed by the visual pigment to quanta incident on the retina (0.23).

Our measurements are in agreement with this estimate. Disc luminance for the experiments with the 5,461 A irradiation was about 110 equivalent lux (photopic) or 35 candelas per square meter at 2.5 log units above a-wave threshold while the thermophile measurement indicated approximately 5 µw per square centimeter to be incident on the cornea at this level of irradiation. When the measured luminance is converted to retinal irradiance the result is 6 µw per square centimeter on the basis of the relationship (cf. Dodt and Echte)

\[ E_e = K^{-1} \cdot L \cdot D^2 \cdot S \cdot f \]

where \( E_e \) is the retinal irradiance, \( K \), the luminous efficiency of light for the standard observer, \( L \), the measured luminance, \( D \), the refractive power of the rat's eye (2.75 cm.⁻¹), \( S \), the pupillary area (assumed 0.20 cm.²) and \( f \), the fraction of light incident on the cornea transmitted through the ocular media (0.8). This value of 6 µw per square centimeter for the 5,461 A is equivalent to about 4 µw per square centimeter of 5,000 A irradiation when related to the spectral ERG sensitivity of the rat's eye.

In the experiments with the green zirconium light (around 5,000 A), disc luminance at 2.5 log units above the a-wave threshold was about 50 equivalent lux, corresponding to a retinal irradiation of 9 µw per square centimeter (computed as above). Thermophile readings gave a value of 11 µw per square centimeter.

Considering the data presented and the possible errors in measurement which may mainly be introduced by the determination of a-wave threshold, the minimally effective retinal irradiation applied for several hours at a high body temperature (cf. Fig. 5) is estimated to be about 1 µw per square centimeter for a wavelength of 5,000 A.

It is obvious that retinal irradiation with the doses found to be effective cannot produce a significant rise in retinal temperature. Even under the assumption that the retina is a perfectly thermoisolated tissue trapping all incident light, several hours of irradiation would be required to raise its temperature by 1° C. with a light intensity of 1 to 10 µw per square centimeter.

Retinal rhodopsin concentration was determined in order to obtain another correlate of the effect of light and an additional measure of light intensity. Exposure time was generally shorter than required to pro-
duce an irreversible effect but long enough to assure that the measurement was representative of the physiological steady-state effect of light on rhodopsin. The eyes were removed within 5 to 10 minutes after exposure and rhodopsin determined by conventional technique. Although a satisfactory correlation between the effects of light on rhodopsin and cellular integrity has not been achieved so far, it is clear that all intensities which were actually proved to be damaging also produced substantial rhodopsin bleaching physiologically. For instance, rhodopsin concentration was on the average slightly less than 30 per cent that of the dark-adapted state when the unanesthetized and unrestrained animals were in the illuminated chambers of Procedure No. 1 for one hour at a normal body temperature. Exposure to the green zirconium light of 11 μW per square centimeter (see above) reduced retinal rhodopsin on the average to 35 per cent that of the simultaneously measured controls. Body temperature was elevated to 104° F. during light exposure in these experiments but about the same bleach seemed to occur when the temperature was in the physiological range. However, a reduction in light intensity by 1 log unit which appears to render it ineffective for producing damage (cf. Fig. 5) was associated with rhodopsin concentrations within the range of variations of the dark-adapted controls. It thus seems that the range of the damaging intensities coincides with that for a measurable effect upon total rhodopsin content. Cone's data lead to the same conclusion. According to his measurement (cf. Fig. 2) a light intensity of 2.5 log units above a-wave threshold (3.5 μW per square centimeter of 5,000 Å which agreed well with our measurements for 5,640 Å radiation) produces about 30 per cent bleaching whereas 1 μW per square centimeter of the same radiation, assumed to be very near the minimum effective dose, reduces rhodopsin concentration by less than 10 per cent.

**Action spectrum of the damaging effect.**

A first indication of the spectral efficiency of light for producing the irreversible damage was obtained by comparing effects when transparent plastics of different colors had been interposed as a wall between the fluorescent bulbs and the exposure chamber (Procedure No. 1, see Methods). In comparison to the routinely used green plastic filter (No. 2,092), a blue (No. 2,045) and a red (No. 2,444) filter were employed. Within the spectral emittance range of the fluorescent bulbs, the peak of transmission for No. 2,092 was at around 530 mμ, and that of No. 2,045 around 445 mμ; No. 2,444 passed all radiation above 600 mμ. Each experiment was run simultaneously with the three “colored” chambers in use in addition to a “dark” chamber. Inside each chamber the temperature was adjusted to the same level; it varied between 28 and 32° C. among experiments. Exposure time was between 24 and 48

<table>
<thead>
<tr>
<th>Table VI</th>
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<tr>
<td>Transmission range of filter (mμ)</td>
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<tr>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Relative irradiance (w./cm.²)</td>
</tr>
<tr>
<td>Relative scotopic efficiency</td>
</tr>
<tr>
<td>a-wave amplitude† (mean ± SD)</td>
</tr>
<tr>
<td>b-wave amplitude† (mean ± SD)</td>
</tr>
</tbody>
</table>

*Approximate limit of emission of the fluorescent bulbs.
†In arbitrary units (100 = mean of control amplitudes).
‡Significantly (p < .001) different from control.
hours; the ERG was tested 3 to 5 days after exposure.

As shown in Table VI there was no difference between the effects of green and blue light. For each of these lights, however, the a-wave was irreversibly reduced in amplitude by 80 per cent in comparison to the control animals (dark chamber), and the b-wave by about 70 per cent. In contrast, red light was ineffective despite the fact (Table VI) that measured irradiance was as high as for the green and blue light.

The ineffectiveness of the red light suggested that the damaging effect of light depended on its absorption by the visual pigment. In order to test this hypothesis, monochromatic lights from the Grating Monochromator were adjusted in intensity by neutral filters so as to be equally strong in eliciting the ERG when applied in form of a 100 msec. flash to a dark-adapted animal. This equalization was attempted for ERGs of various forms but in practice there was always some discrepancy between the spectral efficiency of weak stimuli compared to strong ones. It was decided to adjust all tested wavelengths so that they produced exactly the same ERG at the intensities which were actually used to compare the damaging effect.

During stimulus equalization the animals (albino rats) were in exactly the same position and under the same condition as during the damaging exposure, except that their body temperature was not artificially raised. Equalization was such that a change in intensity by 0.2 log unit produced a clearly different ERG compared to that of the original intensity or the adjusted intensity of another wavelength. This test for equal ERG stimulation was performed on several occasions throughout the course of the experiments.

The wavelengths selected for testing were the mercury lines 4,348/4,358, 5,461, 5,770/5,790 and the relatively weak line near 4,950 A. Compared with the data by Dodt and Echte and Cone on the spectral sensitivity of the b-wave of the dark-adapted albino rat, our relative intensities for equal ERG stimulation deviated from the average of their values by no more than 0.15 log unit, except for 5,778 A which in our series was by 0.25 log unit relatively less effective in stimulating the ERG than their data would indicate.

Essentially two types of experiments were performed. In the one type (Experiment No. 1, Fig. 6) the exit slit was focused upon the cornea and use made of the light-scattering property of the albino eye. Exposure time was 1½ hours; body temperature was maintained close to 104° F. In an early series of experiments of this type involving 48 animals, the a-wave of the exposed eye was irreversibly reduced on the average by 46 per cent (±14.4) following exposure to 4,950 A, by 43.5 per cent (±18) following 4,353 A exposure, and by 38 per cent (±20) after exposure to 5,780 A. Data from a later series of experiments with the same type of exposure are plotted in Fig. 6 and labeled Exp. No. 1. In this series, body temperature was carefully adjusted to be near 104.5° F. As shown in Fig. 6, Exp. No. 1a, the three spectral regions had virtually the same effect of about 50 per cent irreversible ERG loss. On the other hand, when the same radiations were applied equal in terms of irradiance (Fig. 6, Exp. No. 1b) the effects produced with 4,353 A and 5,780 A were small (15 and 11 per cent ERG loss, respectively) compared to the damage resulting from the 4,950 A irradiation which in this series of experiments (No. 1b) was of the same intensity as in Exp. No. 1a and produced the same average effect. However, when the 4,950 A irradiation was weakened by a neutral filter of 0.5 density its effectiveness was reduced from a 50 per cent irreversible ERG loss to an average of 18.5 per cent (±10).

In the second type of experiments (Fig. 6, Exp. No. 2) the monochromatic light illuminated an opal glass over a field of approximately 4.5 cm. diameter, 1 cm. in front of the rat's eye. Exposure time was 3 hours and body temperature near or
above 104°F. The three radiations tested were 4,353, 5,461, and 5,780 Å. The relative efficiency of these radiations for damaging the retina approximated that for eliciting the ERG; thus confirming the results obtained with the other technique.

The apparent similarity in the action spectrum of light-induced damage and visual excitation suggests that the damaging effect is initiated by and directly dependent upon the action of light upon the visual pigment or, as an alternative, that a state of light adaptation is necessary for making effective an action of light upon molecules other than rhodopsin. A precisely determined action spectrum of the effect would be needed to choose between these possibilities.

**Different animal strains.** Four different albino breeds were tested and all showed about the same susceptibility to the damaging effect of light. These include in addition to the generally used CDC or CDF strains, the Sprague-Dawley and Wistar rats, and animals of a cross between Sprague-Dawley rats and inbred albinos afflicted with recessive retinal degeneration. The described effect, therefore, cannot be related to an abnormality peculiar for a certain rat breed.

The hooded (pigmented) animals studied were from a Long Evans strain. When tested by Procedure No. 1 they were significantly less affected than albinos although their pupils were maximally dilated by atropine. As shown in Table VII, this was well displayed at an environmental temperature of 36°C, at which temperature exposure time had to be more than doubled (e.g., from 4 to 8 hours) to produce the same effect as in the nonpigmented animals. With exposure times of 2 to 4 hours at a temperature of 39°C, this difference was less apparent. Pigmented heterozygotes (Ss) from a cross between Long Evans and the pigmented strain of blind animals...
Table VII

<table>
<thead>
<tr>
<th>Exposure time (hours)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albino</td>
<td>21 (9)</td>
<td>11 (7)</td>
<td>—</td>
<td>—</td>
<td>20 (15)</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Hooded Ss</td>
<td>90 (8)</td>
<td>74 (8)</td>
<td>54 (10)</td>
<td>33 (8)</td>
<td>38 (8)</td>
<td>27 (6)</td>
</tr>
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Number of animals in parentheses.

Ss, heterozygous for retinal degeneration; these animals have normal retinal histology and normal ERGs.

(hereditary retinal degeneration) were affected by light no differently than the normal, hooded rats (Table VII).

The relatively low susceptibility of the pigmented animals is additional evidence that the damaging effect of light as manifested in our study differs from retinal thermal injury. The lower susceptibility, however, may not have a cellular basis and may mainly be the result of less effective retinal illumination during procedure No. 1 because it was not apparent when monochromatic light was focused upon the pupil. Under this condition no difference between the two kinds of animals was evident.

**Histological manifestations.** The following description of the histological changes derives from experiments during which the animals were exposed to light at a high body temperature.

Figs. 7 to 10 from albino animals illustrate the sequence of the histological changes following an exposure to the green light of Procedure No. 1 at a rectal temperature of about 103°F. One eye of the animals was removed after exposure while the other served for examination by ERG to assure that eyes of equally affected animals were evaluated at various stages of the histological effect.

The photomicrograph of Fig. 7 shows the outer retinal layers of a rat that was exposed to the light for only one hour and the eye removed at the end of exposure. The appearance of the retina is the same as in the sections from control animals fixed and stained in the same way. This normal appearance was typical for all retinas removed immediately after a relatively short exposure which was unlikely to produce an ERG loss exceeding 50 per cent.

In contrast, the retina of Fig. 8 which was fixed at the end of a 4 hour exposure, shows severe degeneration which with shorter exposure developed only after a delay of several hours. While the sensory organelles do not differ strikingly from their control appearance, the outer nuclei seem to be in an early stage of pyknosis suggesting that the visual cells have been damaged beyond repair.10

The most remarkable abnormality at this early stage of the histological effect is displayed by the pigment epithelium. As shown in Fig. 8, the pigment epithelial cells are swollen so that the distance between their apical and basal borders is considerably increased. Their cytoplasm is very lightly stained; their nuclei are rounder and larger, and the chromatin seems as if reduced and/or dispersed over a greater volume; it also seems to be localized preferentially near the nuclear periphery. Hence, both the visual cells and the pigment epithelium appear to be severely affected at about the same time when light exposure is performed at high body temperature.

This is evident throughout the later stages of the histological effect. At 6 hours after exposure (Fig. 9) visual cell pyknosis has increased while the pigment epithelium cytoplasm is in part replaced by large
Fig. 7. Albino rat retina fixed immediately after a one hour light exposure (Procedure 1) at 39°C. The retina does not differ significantly from controls. (Zenker's fixation, hematoxylincosin histological technique, and magnification are the same in this and all other figures.)

Fig. 8. Albino rat retina fixed immediately after 4 hours of light exposure. Chamber temperature during exposure was 39°C. Outer nuclear layer shows pyknosis and edema; the pigment epithelium is drastically swollen.

Vacuoles. In addition, the visual cell layers of the retina are folded in places and fluid has accumulated at these sites between the apical rod layer and the lining of the pigment epithelium. These folds disappear within the next 12 to 24 hours, probably indicating the equilibration in tissue pressures due to the breakdown of intraretinal diffusion and bulk flow barriers. Cellular damage increases concomitantly.
Fig. 9. Albino rat retina 6 hours after light exposure as in Fig. 8. The outer retina shows numerous folds which disappeared within approximately 1 day. Pigment epithelium under these folds is either destroyed or transformed into large vacuoles.

Fig. 10. Albino rat retina 24 hours after exposure to light as in Figs. 8 and 9. The outer nuclear layer is undergoing dissolution with remarkable rapidity and uniformity.

Fig. 10 is a 24 hour section from the same series of experiments. The remnants of the nuclei of the pigment epithelium have vanished at this stage while in the outer nuclear layer the chromatin is distributed in a diffuse and bizarre manner suggesting that all structural components of this retinal region are in a stage of dissolution. In contrast, the structural outlines of the rods are still apparent; the inner segments, however, have lost their characteristic staining property indicating mito-
chondrial abnormality. The cells of the inner layers seem well preserved but a slight edema appeared to be present extending into the region of the inner limiting membrane.

The final outcome of these changes is the complete disappearance of the visual cell layers and of the pigment epithelium. Bruch's membrane remains as a border between retina and choroid; likewise, the outer limiting membrane can be distinguished over large areas. The inner layers are comparable in appearance to that of a normal retina or one where the visual cells have disappeared as a consequence of the effects of poisons or as the result of a genetic abnormality. The light-damaged retinas differ, however, at a late stage from other abnormal retinas by the frequent occurrence of cysts, such as are illustrated in Fig. 11. Their location varied and included practically all preserved layers of the retina. They were mostly seen in retinas which had been severely damaged as the result of prolonged exposure (Procedure No. 1) at a high body temperature.

Pigmented animals showed the same sequence of changes except that they developed with a slower rate (Fig. 12A). Phagocytotic cells accumulating the melanin of the destroyed epithelium and forming small clusters were evident 72 hours after exposure near the outer border of the retina and remained in this position for up to 2 weeks. Thereafter, they seemed to disappear. Fig. 12B gives an example of the size and location of these pigment clumps.

Many retinas were examined several days or weeks after an exposure which resulted in partial ERG loss. When the animals had been unanesthetized and unrestrained (Procedure No. 1) the damaged area was found preferentially within the nasal region of the retina which one would expect to be more effectively illuminated than the temporal half of the retina. The maximal effect, however, was in the midperiphery and not in the central region, suggesting

Fig. 11. Albino rat retina 21 days after exposure to light as in Figs. 8 to 10. Pigment epithelium and visual cell layers have disappeared. The preserved layers of every section of these retinas in which practically the whole visual cell population has been destroyed (no ERG response), display cysts as illustrated. They are also found in the inner plexiform layer and they number 5 to 10 in each section.
that the cellular susceptibility to light is not evenly distributed over the retina.

In retinal zones bordering a severely affected area, the visual cells or the pigment epithelium could be preferentially affected. In the first case the visual cells were destroyed while the underlying pigment epithelium was preserved; in the second case, the pigment epithelium had disappeared while the visual cell nuclei had survived, although outer and inner segments had degenerated, giving this part of the retina an appearance as typically seen after iodate poisoning.11
Fig. 13. Albino rat retina 3 days after a one hour exposure to light at 36° C. Note the two mitotic figures at the outer border of the inner nuclear layer. There were 7 mitotic figures in this section, all in exactly the same location and near those illustrated.

In some sections of affected retinas we were surprised to find mitotic figures within the inner nuclear layer near its outer border as shown in Fig. 13. They always occurred in exactly the same location, and when present in one section involved a row of more than 5 cells separated from each other by about 3 to 7 bipolar cells. The origin of these cells has not been determined but they may be cells normally present in this layer. These mitotic figures were found only 2 to 3 days after exposure when the visual cells of the same region were undergoing rapid lysis.

The histological appearance of the retina after exposures to relatively weak light at a normal body temperature for several days has not yet been studied extensively. Preliminary observations seem to indicate that under these conditions pigment epithelial damage may not be apparent despite extensive damage of the visual cells and irreversible ERG loss.

Discussion

It was the intent of this study to ascertain first of all that light genuinely damages the rat retina and that it does so by a mechanism other than thermal injury.

This seems to have been achieved. The paradox also seems to have been resolved that laboratory rats with the exception of a special strain show ordinarily normal retinal histology although they have been reared generally in an environment uncontrollable with respect to light. As was shown, the damaging effect of light is extremely dependent upon body temperature and occurs most rapidly and extensively when body temperature is elevated above normal. At a normal body temperature ordinary laboratory illumination apparently is either not strong enough or is not maintained continuously for a sufficient length of time to induce an irreversible effect.

It is reasonable to assume that the damage as manifested by the destruction of visual cells and pigment epithelium and the persistent loss in ERG amplitudes is the result of a chain of reactions of which the last ones only are irreversible because they are beyond the reach of mechanisms of repair. The task is to analyze this sequence and to determine its kinetics step by step. At the present time no more than a few general statements are possible. It is clear that light above a certain intensity initiates and sustains the reaction sequence,
and that this probably requires its absorption by rhodopsin or a rhodopsin-like visual pigment. However, once the reactions have reached a certain point or one of the reactions a certain magnitude, the process can continue in the dark even while retinal excitability recovers from light similarly as in physiological dark adaptation. The damaging reaction, therefore, appears to be a dark reaction with a slow rate constant. Furthermore, the extreme and unusual dependency of the damaging effect upon temperature might best be considered a property of one of the late reactions which are crucial in determining the irreversible effect. Finally, for the understanding of the mechanism of the effect it must be considered significant that the damage involves both the visual cells and the pigment epithelium of the same retinal region when body temperature is elevated during the exposure to light. Hence, under the assumption that the damaging light acts primarily upon the visual cells, there must exist some mechanism of ready transfer of one of the reaction products from visual cell to pigment epithelial cell which enables the reaction to proceed in both cells simultaneously.

It would be premature at the present time to consider in detail possible mechanisms which might be responsible for the damaging effect. It seems appropriate, however, to list briefly several hypotheses which were considered during this study, and which to some extent influenced the experimental design.

In many biological systems, destructive effects of visible light (often called photodynamic actions) are thought to be photosensitized oxidations initiated by the action of light upon a dye or a natural pigment. The dye or pigment acts catalytically in this chain reaction which ultimately leads to the reaction of an activated substrate with free oxygen to form a peroxide. Exploratory experiments which could support the hypothesis that the damaging effect of light upon the retina is analogous to photosensitization have so far yielded no convincing results. This includes tests on vitamin E-deficient animals, on the influence of environmental oxygen pressure, and on retinal lipid peroxide formation subsequent to exposure to light. Moreover, the participation of the pigment epithelium in the damaging reaction is difficult to reconcile with the assumption that light acting upon the rhodopsin of the visual cells initiates a chain reaction of this kind. However, the action spectrum of the damaging effect has not yet been determined with the precision necessary to exclude the presence of another pigment as the agent of the light-induced effect.

Second, it might be suggested that intense and prolonged light adaptation adversely affects a metabolic pathway essential for the maintenance of cellular integrity especially at a high temperature. For instance, it is known that retinal oxygen consumption is depressed by steady exposure to light and conceivably this might be associated with changes in the concentration or distribution of a metabolite that controls intracellular membrane functions. In comparison to metabolic poisons one would expect in this case that the histologically evident changes in cell structure occur with considerable delay after effects upon excitatory function such as are recorded by the ERG have become manifest. Characteristically, however, the effect of light at a high temperature leads very rapidly to histological changes, in fact more rapidly than has been observed so far with poisons like iodoacetate or with anoxia or with exposure to a high oxygen pressure.

A third possibility is that light exposure yields a potentially toxic photoproduct against which the rat retina is poorly protected. There has accumulated during recent years considerable evidence that vitamin A adversely affects the integrity of membrane systems. Unpublished observations by J. G. H. Schmidt and W. K. Noell.
vitamin A is a potent hemolytic compound when added to a suspension of red cells\textsuperscript{19}; it is a mitochondrial swelling agent in certain tissues\textsuperscript{20}; it makes cultured fibroblasts to swell drastically\textsuperscript{21}; and it induces the massive release of proteolytic enzymes from isolated lysosomes.\textsuperscript{18} Furthermore, its effects upon red cells and lysosomes are very temperature dependent and are exerted mainly at a temperature above 35 to 37° C. The doses of vitamin A effective upon lysosomes are also very low and probably just within the range of the vitamin A concentration to be expected in a thin retinal compartment after the bleaching of rhodopsin. Proteolytic enzymes (50 percent of the total activity) are released at 37° C. from a lysosomal fraction of liver tissue by 0.25 μg of vitamin A per milligram of the original tissue,\textsuperscript{22} while according to Dowling\textsuperscript{23} about 0.2 μg of vitamin A is found per rat retina (40 mm\textsuperscript{2} area) during intense exposure to light. However, this analogy also poses difficulties. Most of the vitamin A formed in the rat retina during light exposure apparently is transferred within one hour to the pigment epithelium so that the visual cell concentration of vitamin A is relatively low during a continuous exposure except for a brief period after the start of light.\textsuperscript{23} In addition, almost all vitamin A in the pigment epithelium was found esterified\textsuperscript{24} but vitamin A esters added to a preparation of lysosomes are ineffective.\textsuperscript{18} Nevertheless, the similarities between the toxic effect of vitamin A and that of light are so great that they need to be explored in future experiments.

The damage produced by light has so far been observed only in the laboratory rat and it may not apply to other species nor to a wild-type rat strain. Species differences in the retinal response to poisons are a common laboratory experience. In the laboratory rat, one of the reactions initiated by light may be relatively strong, perhaps because of a deficiency in a mechanism that could oppose it. Similarly, there may exist in human disease a condition which causes light to have an as yet unknown adverse effect.

\textbf{REFERENCES}


Discussion

Dr. J. E. Dowling. Mr. Robert Mittenthal in our laboratory has been performing similar experiments and we can confirm Dr. Noell's results that bright light causes destruction of visual cells in an albino rat retina in a relatively short time. All of our experiments have been done at room temperature, and, with the fluorescent lighting arrangement we have used, it takes about 48 hours of continuous light for permanent retinal damage to occur. Six to eight days of continuous light will completely destroy almost all visual cells in the retina.

I have two comments for Dr. Noell. First, we have found that in the first 24 hours of continuous light, the eyes of the rats become deeply reddened and often they protrude conspicuously as though the orbital tissues have swollen. I wonder if Dr. Noell has observed the same thing and whether he has any comment on this.

Second, we have not been impressed that the

![Fig. 1. Retinal histology following prolonged light exposure. a, Control retina. b, Retina from animal kept in light for 5 days and then allowed to recover in darkness for 2 months. About three quarters of the visual cells in this area of the retina have been destroyed but the overlying pigment epithelium looks intact. Bipolar and ganglion cells also look normal. c, Retina from an animal kept in light for 8 days and allowed to recover in the dark for one month. Here the visual cells have been entirely destroyed, and pigment epithelial cells have broken away from Bruch's membrane and appear to be migrating through the retina. Again the bipolar and ganglion cells look intact.](image-url)
pigment epithelium shows any changes until the visual cells adjacent to the pigment epithelium are entirely destroyed. Then, the pigment epithelial cells do break off from Bruch’s membrane and migrate through the retina. However, it appears to us that as long as some visual cells remain in an area of the retina, the pigment epithelium overlying these cells remains intact. This is illustrated in Fig. 1.

Dr. Noell. We have noted signs of ocular irritation only in a few (discarded) experiments at the very beginning of our study two years ago. Since then a green filter is interposed between light source and animal in our standard (No. 1) procedure and a less intense source used than initially.

We have studied histologically numerous eyes after short or long exposure at an elevated body temperature; severe damage of the pigment epithelium in albino as well as hooded animals was evident whenever the adjacent visual cells were severely affected. The only exception we observed was the border zone of a severe lesion where the pigment epithelium could be preserved several days or weeks after exposure while the visual cells were reduced in number. However, we have also seen the opposite, that the pigment epithelium in the border zone between affected and unaffected regions was destroyed while most visual cell nuclei had survived as in case of a primary pigment epithelial damage. On balance we concluded that the pigment epithelium and the visual cells are about equally sensitive to the damaging effect of light, at least at a high temperature, in contrast to our experiences with chemical poisons which affect primarily the one or the other cell population depending upon the mechanism of action.

We have no convincing evidence on pigment cell migration either for the damaging effect of light or for responses to poisons; but on occasion we have seen pigment epithelial proliferation in response to a chemical poison.