Fig. 3. Oscillogram of a Ca++-induced spike from chick retinal pigment epithelium in culture. The grid indicates 10 millivolts vertically and 200 milliseconds horizontally. Positively is upward.

The concentration of Ca++ in the culture media less than 0.01 millimoles. Since the solution has to be applied near the cells in question, the local concentration required to induce these responses must be higher than this. An order of magnitude estimate of this triggering concentration can be obtained by assuming that the pigment epithelium cell membrane approximates a K+ electrode, an assumption supported by the fact that K+ is very effective in depolarizing these cells (Table I). The depolarization produced by 10 microliters of 0.5 M KCl was 36 ± 12 mv., which would be produced by about a 20 to 40 millimole increase in K+ outside the cell. The triggering concentration of Ca++ is probably near this range.

It would be interesting to know whether this curious phenomenon induced by Ca++ plays any role in the normal function of retinal pigment epithelium. As far as we know spikes have only been found in nerve and muscle and never in any epithelial cell.

The hyperpolarizing wave that precedes the spikes bears some resemblance to the c-wave of the electroretinogram which is a hyperpolarizing potential induced in intact retinal pigment epithelium by light impinging on the photoreceptors. Steinberg and Miller have suggested that the light-induced Na+ conductance change in the photoreceptors leads to a local reduction of extracellular K+ and hence to a hyperpolarization of the retinal pigment epithelium. Some direct support for this hypothesis has recently been obtained. If Ca++ were released from the photoreceptors by light, as has also been suggested, the late hyperpolarization it produces on pigment epithelial cells could also contribute to the c-wave of the electroretinogram.

We would like to thank Drs. Fernando de Melo and Dr. Marshall Nirenberg for their assistance in this project.

REFERENCES
6. Oakley, B., and Green, D. G.: The ionic basis of the c-wave of the electroretinogram, ARVO meeting abstracts, 1975, p. 5.
Table I. Number of photoreceptor cells* in sections of retinas from Fischer rats in different lighting conditions

<table>
<thead>
<tr>
<th>Lighting condition</th>
<th>CyL</th>
<th>CyL</th>
<th>CL</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age into CL:</strong></td>
<td></td>
<td></td>
<td>7 weeks</td>
<td>7 months</td>
</tr>
<tr>
<td><strong>CL duration:</strong></td>
<td></td>
<td></td>
<td>54 days</td>
<td>31 days</td>
</tr>
<tr>
<td><strong>Age at fixation:</strong></td>
<td></td>
<td></td>
<td>3.4 months</td>
<td>8 months</td>
</tr>
<tr>
<td><strong>Type of fixation:</strong></td>
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<td></td>
<td>Perfusion</td>
<td>Perfusion</td>
</tr>
<tr>
<td><strong>Age at fixation:</strong></td>
<td></td>
<td></td>
<td>10.4 months</td>
<td>3.4 months</td>
</tr>
<tr>
<td><strong>Type of fixation:</strong></td>
<td></td>
<td></td>
<td>Perfusion</td>
<td>Perfusion</td>
</tr>
<tr>
<td><strong>CL duration:</strong></td>
<td></td>
<td></td>
<td>54 days</td>
<td>31 days</td>
</tr>
<tr>
<td><strong>Age at fixation:</strong></td>
<td></td>
<td></td>
<td>3.4 months</td>
<td>8 months</td>
</tr>
<tr>
<td><strong>Type of fixation:</strong></td>
<td></td>
<td></td>
<td>Perfusion</td>
<td>Perfusion</td>
</tr>
<tr>
<td><strong>CyL</strong></td>
<td></td>
<td></td>
<td>4.4 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td><strong>Rods</strong></td>
<td></td>
<td></td>
<td>75.0 ± 4.2</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td><strong>Per cent cones</strong></td>
<td></td>
<td></td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>CL</strong></td>
<td></td>
<td></td>
<td>10.4 months</td>
<td>3.4 months</td>
</tr>
<tr>
<td><strong>Age at fixation:</strong></td>
<td></td>
<td></td>
<td>10.4 months</td>
<td>3.4 months</td>
</tr>
<tr>
<td><strong>Type of fixation:</strong></td>
<td></td>
<td></td>
<td>Perfusion</td>
<td>Perfusion</td>
</tr>
<tr>
<td><strong>CyL</strong></td>
<td></td>
<td></td>
<td>2.6 ± 0.4</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td><strong>Rods</strong></td>
<td></td>
<td></td>
<td>161.1 ± 10.6</td>
<td>14.3 ± 2.2</td>
</tr>
<tr>
<td><strong>Per cent cones</strong></td>
<td></td>
<td></td>
<td>1.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M. per 180 μm length of retina; counts based on fifteen 180 μm lengths, five consecutive lengths in each of three sections, beginning in each section either about 400 μm from the optic disc and progressing peripherally (posterior retina) or at the ora serrata and progressing centrally (peripheral retina). CL, continuous light; CyL, cyclic light.

Table II. Number of photoreceptor cells* in sections of retinas from Fischer rats

<table>
<thead>
<tr>
<th>Lighting condition</th>
<th>CL</th>
<th>CL</th>
<th>CL</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age into CL:</strong></td>
<td>7 weeks</td>
<td>7 weeks</td>
<td>7 months</td>
<td>7 months</td>
</tr>
<tr>
<td><strong>CL duration:</strong></td>
<td>178 days</td>
<td>264 days</td>
<td>70 days</td>
<td>147 days</td>
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<tr>
<td><strong>Age at fixation:</strong></td>
<td>7.6 months</td>
<td>10.4 months</td>
<td>9.3 months</td>
<td>11.9 months</td>
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<tr>
<td><strong>Type of fixation:</strong></td>
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<td>Perfusion</td>
<td>Perfusion</td>
<td>Perfusion</td>
</tr>
<tr>
<td><strong>CyL</strong></td>
<td>20.2 ± 2.0</td>
<td>14.2 ± 2.0</td>
<td>42.2 ± 1.6</td>
<td>31.8 ± 5.0</td>
</tr>
<tr>
<td><strong>Rods</strong></td>
<td>15.6 ± 0.8</td>
<td>8.2 ± 1.8</td>
<td>28.8 ± 1.1</td>
<td>19.2 ± 1.7</td>
</tr>
<tr>
<td><strong>Per cent cones</strong></td>
<td>56.4</td>
<td>63.4</td>
<td>59.4</td>
<td>62.4</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M. per section of retina from optic disc to ora serrata; counts based on five sections. CL, continuous light.

of the degeneration is influenced by the age1 and body temperature2 of the animal, the intensity of illumination,3-4 and the exposure time.2, 3-5 Behavioral studies of albino rats whose retinas were apparently devoid of photoreceptor cells due to exposure to continuous light in the 20 to 70 foot-candle incident illuminance range indicate that these animals are able to perform light-dark and pattern discrimination as well, or almost as well, as normal control animals.6-10

It had previously been thought that all photoreceptors degenerate in RCS rats with inherited retinal dystrophy because none were recognizable by conventional histology in animals of several months of age. In a recent behavioral and cytological study, however, it was found that these rats can discriminate light intensity at ages up to 2 years, and, using cytological procedures that intensify photoreceptor heterochromatin staining, numerous surviving photoreceptor cells were observed.11 In view of these observations, it was of interest to examine the retinas of albino rats maintained in long-term continuous light with the same cytological procedures as used for study of the RCS rats.

Methods. One litter of nine rats, inbred descendants of Cesarean-derived Fischer rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), were born and reared in a 12-hour light-12-hour dark environment in cages illuminated at less than 15 foot-candles and at a room temperature of 24 ± 1° C. The rats were maintained in transparent polycarbonate cages with stainless steel wire-bar covers with food (Charles River 18 RF pellets) and water provided ad libitum. The bedding used was a No. 5 birch cube ("Betta-chip," Northeastern Products Corporation, Warrensburg, N. Y.). At seven weeks of age, six of the rats were transferred to a room with constant fluorescent lighting with illuminance at cage level of about 16 foot-candles. In addition, a lamp containing two 15W fluorescent bulbs (ITT, Daylight No. F15T8/D) was positioned about 70 cm. above the bottom of the cage. The lamp continuously provided an incident illuminance of about 65 foot-candles at the floor of the cage. This illuminance designation does not allow for calculation of the effective retinal illuminance, but it is used to facilitate comparison with published studies on constant light damage. The eyes were taken from two rats after 54 days in constant light, from two rats after 178 days, and from...
two after 264 days. At each time eyes were taken from one littermate control rat kept in cyclic light. Throughout the light exposure the temperature in the cage remained at 24 ± 1°C.

Three additional Fischer rats were exposed with a somewhat higher illuminance level beginning at seven months of age. Fluorescent lights were positioned about 40 cm over a white, opaque, polyethylene cage with an in-cage feeder so that the floor of the cage had a uniform incident illuminance of about 140 foot-candles. Eyes were taken from one rat after 31 days in constant light, from one after 70 days, and from one after 147 days.

Eyes were either enucleated and immersed in fixative or dissected out after vascular perfusion of the rats with fixative (Tables I and II). The eyes were then postfixed in osmium tetroxide, stained en bloc with uranyl acetate and embedded in an Epon-Araldite mixture as described elsewhere. The combined use of glutaraldehyde, formaldehyde, and calcium ions in the initial fixative and the use of en bloc staining with uranyl acetate result in an increased binding of toluidine blue to photoreceptor nuclear heterochromatin.

One to 1.5 mm sections were cut such that the retina from the optic disc to the ora serrata was included in a single section, and selected areas were examined by electron microscopy.

Results.

Rats in cyclic light. The retinas from the control rats maintained in cyclic light were normal (Fig. 1, F). Most of the photoreceptor cells had small nuclei, usually less than 5.5 μm in diameter, with one large, central clump of heterochromatin. These features were typical of rod cells. About 1.5 per cent of the photoreceptor cell nuclei contained multiple small clumps of heterochromatin, were larger than about 5.5 μm, were usually somewhat ovoid in shape and were located in the outer one-third to one-half of the outer nuclear layer (Fig. 1, F and Table I). Cells with these features have been described as cones in the rat retina (see references in 11) and although they do not display typical cone inner and outer segment structure, they will be referred to as cones in this report to distinguish them from the rods.

Rats placed into continuous illumination at seven weeks of age. After 54 days in constant light the outer nuclear layer in most regions of the retinas was reduced to one incomplete row of photoreceptor nuclei (Fig. 1, A). The outer plexiform layer essentially was missing in these areas, and many of the photoreceptor cells appeared to be displaced into the outermost part of the inner nuclear layer. In the apparent absence of inner and outer segments, the identification of the strongly basophilic nuclei as those of photoreceptor cells was supported by their arrangement as a row continuous with an outer nuclear layer comprised of one to two rows of nuclei in a small region of peripheral retina (Fig. 1, B). The photoreceptor cells in this region had remnants of at least some inner segments and an underlying outer plexiform layer. Degeneration and disappearance of photoreceptor cells was more extensive at the posterior pole of the eye than at the periphery (Table 1).

After 178 and 264 days in constant light, although most of the photoreceptor nuclei had disappeared from the retinas (Table II*), some could be seen at the outermost aspect of the inner nuclear layer either as one or two nuclei per field (Fig. 1, C) or in clusters of several nuclei (Fig. 1, D). Occasionally, the nuclei were displaced into the middle or inner part of the inner nuclear layer (Fig. 1, E).

Fewer photoreceptor nuclei appeared to survive in the retinas of rats exposed to constant light for 264 days than in those retinas exposed for 178 days (Table II). Other differences in the retinas at these time points were more gliosis, greater disruption of the inner layers, and increased vascularization of the pigment epithelium from the retinal capillaries in animals with the longer exposure. These features have been described previously4, the only exception was that no anastomoses of retinal and choroidal vessels were

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*Note that because so few surviving cells were present at later intervals, cell counts are expressed as number per retinal section in Table II rather than per 180 μm length of retina as in Table I. The retinal length from optic disc to ora serrata in a three-month-old Fischer rat is approximately 5,000 μm, or 28-180 μm lengths. Thus, the number of cells per section in Table II can be divided by 58 to obtain an approximate number per 180 μm retinal length to compare with data in Table I.

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Fig. 1. A through E. Light micrographs of retinas from Fischer rats after continuous light exposure for different periods of time. A, 54 days of exposure; posterior retina. An incomplete row of photoreceptor nuclei is present. B, 54 days of exposure; peripheral retina. One to two rows of photoreceptor nuclei remain, as do some remnants of inner and outer segments. C, 178 days of exposure. A few single photoreceptor nuclei are present, and the heterochromatin of a presumed rod cell nucleus appears contracted (arrow; cf. with Fig. 1, F). D, 264 days of exposure. A cluster of surviving photoreceptor nuclei is illustrated. E, 178 days of exposure. A photoreceptor nucleus has been displaced or has migrated deep into the inner nuclear layer (arrow). F. Outer nuclear layer of a 3.4-month-old Fischer rat reared in cyclic light. Three cone nuclei (arrows) are present among the rod nuclei. c, capillary; pe, pigment epithelium. 1 to 1.5 μm Epon-Araldite sections. Toluidine blue. All ×960.
Fig. 1. For legend, see opposite page.
observed in the present study. However, 1 to 1.5 mm plastic sections do not lend themselves to extensive serial sectioning which might be required to observe such a feature.

Rods apparently respond more quickly than cones to the damaging effects of continuous illumination because initially they were lost in higher proportion. For example, after 54 days in constant light almost 98 per cent of the rods had disappeared, but only about 60 per cent of the cones were missing from the posterior retina. In the peripheral retina almost 90 per cent of the rods were missing, but few, if any, of the cones had disappeared (Table I). This disproportionate loss of rods resulted in the progressive increase in the percentage of cones, such that after 178 and 264 days of constant light exposure about 60 per cent of the photoreceptors were classified as cones (Table II).

The classification of cell types based on nuclear morphology was relatively simple in the 54-day retinas, but became more difficult at later intervals. At the later intervals most of the cells became ovoid instead of rounded, and the central cump of heterochromatin of most rods appeared contracted (Fig. 1, C). Some small nuclei counted as rods may have been tangential sections through cone nuclei. Thus, the percentage of cones in the 178- and 264-day animals may be underestimated.

The retinas of animals exposed 178 and 264 days to continuous illumination were also examined by electron microscopy, and features of the surviving photoreceptors at both intervals were similar. In residual outer plexiform layers, photoreceptor synaptic terminals were observed which were not in the plane of section with the parent cell body (Fig. 2, A and B). In other cases, cells with clumped nuclear heterochromatin were found with synaptic ribbons in their perikaryal cytoplasm (Fig. 2, C). In photoreceptor perikarya, conspicuous Golgi complexes, abundant ribosomes, rough endoplasmic reticulum, and ciliary basal bodies (Fig. 2, D), some with ciliary filaments (Fig. 2, E), were usually found, but rod outer segment membranes were never observed. Occasionally, small whorls of membranes were seen between cells; they were not in obvious continuity with any cell type and were probably a degenerative feature. Ribbon and conventional synapses were present in the inner plexiform layer.

Rats placed into continuous illumination at seven months of age. Photoreceptors degenerated slightly faster when older rats were placed in constant light of a somewhat higher illumination (Table I). Other features of photoreceptor degeneration in the older rats were similar to those of the younger animals (Table II).

Discussion. It has been reported that all photoreceptor cells are missing from albino rat retinas following continuous light exposure of 70 foot-candle incident illuminance for 30 days6 or 36 foot-candle incident illuminance for 120 days.16 These retinas may have contained some surviving photoreceptors, perhaps undetected with the cytologic procedures that were employed, because in the present study some photoreceptors survived more than 8.5 months of continuous light at an incident illuminance of 65 foot-candles (Table II). The survival of these cells may have been due to the age of the animals, since the rats were seven weeks old at the start of light exposure. Photoreceptors in seven-week-old rats are more resistant to degeneration than are those in older animals.1 The report of age-dependency,1 therefore, prompted the use of an even higher (140 foot-candle) illuminance level and a cage with white reflective sides in an attempt to augment the destruction of photoreceptor cells in seven-month-old rats. Under these conditions most of the photoreceptor cells had disappeared after 31 days of exposure (Table I), but about 50 cells per section still remained after 147 days of exposure (Table II). It is also possible that differences in rat strains in the various studies might have influenced photoreceptor survival, although Noell and co-workers2 found that four different albino strains showed about the same susceptibility to the damaging effect of light.

If some photoreceptor cells were, in fact, present in the retinas of rats described in behavioral studies on light-damaged retinas,6-10 these surviving cells may have mediated the visually guided behavior. In the present study the surviving cells were synaptically related to post-synaptic processes of the inner nuclear layer, and the synaptic cytoarchitecture of the inner plexiform layer appeared intact. After long-term constant light exposure, rats showed progressive behavioral performance deficits10 which might reflect either the progressive loss of residual photoreceptor cells as described in the present report or subtle synaptic rearrangements in the visual system.18

Although it has been suggested that other retinal cells may be responsible for the behavioral response to light in the rats with light-damaged retinas,9 the simplest hypothesis is that some surviving photoreceptor cells mediate the behavioral responses. If so, the transduction mechanism remains unclear since outer segments are the first part of the photoreceptor cells to be damaged by light,5, 9 and the cells in the present study showed no outer segments. Possibly photoreceptive molecules reside in the plasma membrane or other parts of the surviving photoreceptor cells.

It is thought that the rat retina contains both rods and cones (see references in 11). Cones in albino rats appear to be more resistant than rods to destruction by constant light. The reason for the
Fig. 2. Electron micrographs of retinas from Fischer rats after continuous light exposure for either 178 or 264 days. A and B, photoreceptor synaptic terminals located in the residual outer plexiform layer not in the plane of section with the parent cell bodies. Both x22,900. C, a surviving photoreceptor cell is illustrated with synaptic ribbons in its cytoplasm. x22,900. D, basal bodies (arrows) are present in a surviving photoreceptor cell. p, pigment epithelial cell processes. x17,540. E, A basal body in another photoreceptor cell has ciliary filaments extending from it (arrow). x20,260.
difference in sensitivity between rods and cones is not clear, but may depend upon a basic difference in the metabolism of the two cell types. Thus, constant light joins a number of destructive agents that affect rods earlier or to a greater extent than cones, including iodoacetate, X-irradiation, and the mutant genes responsible for inherited retinal degeneration in the mouse (Carter and LaVail, in preparation), inherited retinal dystrophy in the rat (LaVail, in preparation), progressive retinal atrophy in the Irish Setter and Norwegian Elkhound (see references in 17), and retinitis pigmentosa in man.

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From the Department of Neuropathology, Harvard Medical School and the Department of Neuroscience, Children's Hospital Medical Center, Boston. This investigation was supported in part by United States Public Health Service Research Grant EY-01202 and Career Development Award EY-70871 from the National Eye Institute. Submitted for publication Aug. 18, 1975. Reprint requests: Dr. Matthew LaVail, Department of Neuroscience, Children's Hospital Medical Center, 300 Longwood Ave., Boston, Mass. 02115.

Key words: light-induced photoreceptor degeneration, rods and cones, albino rats.

REFERENCES


Effect of the ocular media on the main wavelengths of argon laser emission. OLEG POMERANTZEEF, HIROSHI KANeko, R. H. DONOVAN, C. L. SCHEPENS, AND J. W. MCMEEL.

The purpose of this study is threefold: (1) spectrally selective scattering of light in the eye media has been reported. We show that it affects the argon green wavelength (514.5 nm.) and the argon blue wavelength (488 nm.) differently during photocoagulation. (2) The presence of yellow pigment has been shown to absorb more strongly the argon blue than green. We show that it enhances the difference in effect due to scattering alone. (3) In the human eye, yellow staining and scattering of the media are both increased with age. The influence of this factor is noticeable when comparing the results of photocoagulation in old and young patients.

Materials and methods. Eight owl monkeys were selected for the study of scattering media devoid of yellow pigment. Two macaques were utilized for study of the effect of yellow pigment.