Differential effect of the rd mutation on rods and cones in the mouse retina

Louvenia D. Carter-Dawson,* Matthew M. LaVail,** and Richard L. Sidman

The retinas of rd/rd C57BL/6J-rd le mice have been examined by light and electron microscopy to determine whether rod cell degeneration precedes cone cell degeneration. In all regions of the eye, a rapid rod degeneration precedes a much slower cone degeneration. Only about 2% of the rods remain in the posterior region at postnatal day 17, and none by day 36. By contrast, at least 75% of the cone nuclei remain at day 17. Although most of these slowly disappear, about 1.5% of the original population of cone nuclei in the posterior retina is still present at 18 months of age. A central to peripheral temporal gradient of degeneration exists, such that some rod nuclei persist in the far periphery up to day 47, but none is found at day 65. About 5% of the cone nuclei are still present in the far periphery at 18 months of age.

Key words: inherited retinal degeneration, mouse, rods, cones.

The existence of an inherited retinal defect leading to the rapid degeneration of photoreceptor cells has been known in mice since 1924 from the work of Keeler. The disorder is caused by the autosomal recessive gene, retinal degeneration (gene symbol, rd). The disease is present in a relatively large number of inbred mouse strains and is genetically iden-
Carter-Dawson, LaVail, and Sidman


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Table I. Surviving photoreceptor cells* in sections of retinas from normal and rd/rd mutant mice

<table>
<thead>
<tr>
<th>Retina</th>
<th>Normal (+/rd le)</th>
<th>Mutant (rd le/rd le)</th>
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<tr>
<td></td>
<td>P19†</td>
<td>P120</td>
</tr>
<tr>
<td>Posterior:</td>
<td></td>
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</tr>
<tr>
<td>Rod cells</td>
<td>249.7 ± 3.0</td>
<td>218.9 ± 4.3</td>
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<tr>
<td>Cone cells</td>
<td>7.7 ± 0.5</td>
<td>6.8 ± 0.6</td>
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<tr>
<td>% cone cells</td>
<td>3.0</td>
<td>3.0</td>
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<tr>
<td>Peripheral:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rod cells</td>
<td>251.6 ± 4.5</td>
<td>238.3 ± 4.4</td>
</tr>
<tr>
<td>Cone cells</td>
<td>7.3 ± 0.6</td>
<td>5.9 ± 0.4</td>
</tr>
<tr>
<td>% cone cells</td>
<td>2.8</td>
<td>2.4</td>
</tr>
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</table>

*Mean ± S.E.M. per 180 μm length of retina; counts based on 20-180 μm lengths, 5 consecutive lengths in each of 4 sections, beginning in each section either 400 μm from the optic disc moving peripherally (posterior retina) or 400 μm from the ora serrata moving centrally (peripheral retina).
†Postnatal day (denotes age at fixation).
Not included in the analysis because of the difficulty in distinguishing the incompletely differentiated rod and cone nuclei in the peripheral retina at this age.

whereas the cones degenerate much more slowly, and some survive up to at least 18 months of age.

Materials and methods

The mice studied were of the C57BL/6J-rd le strain which carries the closely linked autosomal recessive genes retinal degeneration (rd) and light ear (le). Matings were made between ++/rd le × rd le/rd le mice to give progeny of the same genotypes in approximately equal numbers. These matings provide in the same litter normal heterozygote and homozygous mutant animals that are genetically identical to each other except for the selected segment of chromosome 5 and are congenic with the standard C57BL/6J strain. All mice were maintained at a temperature of 23 ± 1° C in a 12 hr light, 12 hr dark environment at approximate cage illumination of less than 20 footcandles. They were fed Purina Formulab Chow (Ralston Purina Co., St. Louis, Mo.) and given water ad libitum.

The eyes were enucleated under ether anesthesia between 17 days and 18 months of age. The corneas were punctured, and the eyes were fixed by immersion overnight in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4. Subsequently, the eyes were processed as described elsewhere for light microscopic examination of 1 to 2 μm thick sections embedded in an Epon-Araldite mixture, including en bloc staining with uranyl acetate. This procedure facilitates photoreceptor identification by enhancing nuclear heterochromatin staining. The plane of section extended along the vertical meridian from the optic nerve head in the posterior retina to the ora serrata in the far peripheral retina.

Counts of photoreceptor cells were made both at the posterior pole of eye and in the far periphery. Five fields, each 180 μm in length, were examined, beginning 400 μm from the optic nerve head and moving peripherally, and another five fields were examined beginning 400 μm from the ora serrata and moving posteriorly. The number of rod and cone cells was recorded in each region, and the mean, standard error of the mean, and the percentage of each cell type were calculated.

For electron microscopy, rd/rd mutant mice 16 and 36 days of age were fixed by cardiac perfusion with a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer for 2 to 3 min, followed by a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M buffer for 6 min. The eyes were dissected, and the tissue was then processed in the same way as the enucleated eyes.

Results

Light microscopic analysis. In the normal mouse retina, cone nuclei are readily distin-
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<table>
<thead>
<tr>
<th>P47</th>
<th>P65</th>
<th>P120</th>
<th>18 mo</th>
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<tr>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2.2 ± 0.3</td>
<td>0.8 ± 0.2</td>
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<tr>
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<th>P47</th>
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<tr>
<td>0.1 ± 0.0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>2.8 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>96.6</td>
<td>100</td>
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guished from rod nuclei by light microscopy following our cytological protocol. Cones have one to several central clumps of heterochromatin surrounded by a substantial amount of lightly staining euchromatin (Fig. 1). The apparent multiple clumps of heterochromatin are actually one lobulated, polymorphic clump. Cones have one to several central clumps of heterochromatin surrounded by a substantial amount of lightly staining euchromatin (Fig. 1). The apparent multiple clumps of heterochromatin are actually one lobulated, polymorphic clump. The rod nuclei, in contrast, contain a central clump of heterochromatin that almost fills the nucleus, and the small amount of peripheral euchromatin is usually more deeply stained than that of the cone nuclei (Fig. 1).

Examination of sections of posterior retina from the heterozygous control animals showed no significant difference in the percentage of total rod and cone cells between a 19-day-old animal and a 120-day-old animal (Table I). However, a decrease from 250 ± 3 to 219 ± 4 was seen in the absolute number of cells per unit length in the older animals. This is to be expected, since the thickness of the retinal layers decreases as the size of the eye increases17 and no additional cells are generated.18

In the posterior retina of the rd/rd mouse about 98% of the rod nuclei had degenerated by 17 days of age. Less than 5 rods/180 /μm length of retina remained in the mutant retina at postnatal day 17, whereas the retina of the normal heterozygous animal at 19 days of age contained about 250 rods/180 μm (Table I). In contrast, most of the cone nuclei were still present at 17 days of age (Table I). With increasing age (Fig. 2), more rod cells and a smaller percentage of cone cells degenerated, leaving only cone cells at postnatal day 36 and at subsequent ages (Table I).

Positive identification of every surviving nucleus in a given section was difficult, so that it is possible that a few rods persisted past day 36; however, none was found with a completely normal rod chromatin pattern.

A significant decrease in the mean number of cone nuclei was seen in the posterior retina possibly as early as postnatal days 21 to 26. Approximately one half of the cone cells was lost between 17 and 36 days of age, and at 18 months only about 1.5% of the original cone population remained in the posterior retina (Table I). It was difficult to obtain completely unequivocal counts of cone nuclei. Macrophages had invaded the outer nuclear layer by this age,19 and their nuclear chromatin pattern is similar to that of the cone nuclei in some sections. To be sure that only cone nuclei were counted, only those nuclei with a distinct central clump of heterochromatin were recorded. Thus a number of cells which were, in fact, cones were not counted, and the mean number recorded was probably an underestimate. It should be noted that the number of uncounted cells at day 17 was substantial, perhaps twice the number of the counted cells.

Rod nuclei survived longer in the peripheral retina than in the posterior retina. By 36 days of age, no rod nuclei were seen in the posterior retina, but some still persisted in the peripheral retina through at least 47 days (Table I). The delay in degeneration of rod nuclei in the peripheral retina is consistent with earlier observations of Noell27 of a central-peripheral gradient in photoreceptor degeneration, although he reported that the cells degenerated considerably earlier.

Cone nuclei of the rd mutant also survived longer in the peripheral retina than in the posterior retina. Although some nuclei of cone cells were lost as early as postnatal day 21 (Table I), the number remained relatively constant until 65 days of age. At 18 months of age, about 5% of the original population of cone nuclei in the peripheral retina was still surviving (Table I).

Photoreceptor degeneration in the far periphery near the ora serrata appeared to pro-
Figs. 1 and 2. Light micrographs of 1 to 2 μm Epon-Araldite sections of normal (Fig. 1) and rd/rd (Fig. 2) mouse retinas. (Toluidine blue; ×1,200.)

Fig. 1. Normal adult mouse retina. Several cone nuclei (arrows) are shown near the outer limiting membrane. *is*, Inner segments; *os*, outer segments; *pe*, pigment epithelium; *r*, rod nuclei.

Fig. 2. rd/rd retina at 26 days of age. All rod nuclei, outer segments, and apparent inner segments have degenerated, leaving only cone nuclei (arrows) in the outer nuclear layer. These surviving nuclei, with 1 to 3 apparent clumps of heterochromatin, are located adjacent to the pigment epithelium (*pe*).

It proceeded at the same rate in both the superior and inferior retina along the vertical meridian and in both the nasal and temporal retina along the horizontal meridian. This finding is in sharp contrast to the regional differences seen in pigmented rats with inherited retinal dystrophy.15

**Electron microscopic analysis.** It seemed possible that the single, large clump of nuclear heterochromatin in degenerating rods might retract or break up, thereby causing us to misidentify degenerating rod nuclei as cone nuclei by light microscopy. Since we have identified several ultrastructural features that distinguish rods from cones in the normal mouse retina,13 we sought to clarify this problem by electron microscopic examination of the rd/rd mouse retina. We exam-
ined the posterior retina of rd/rd mice at day 16, when approximately equal numbers of rods and cones should be present (Table I), and at day 36, when all the rods should have disappeared (Table I).

At 16 days of age the rod nuclei contained several clumps of heterochromatin in the mutant retina, a feature which is characteristic of normal immature rods. Despite this feature, a distinction between rod and cone nuclei could still be made, since cone nuclei at this age generally contained one small clump of heterochromatin. In addition to the staining properties of the nucleus, features of the synaptic terminals and of the size of the mitochondria served to distinguish rods and cones in the rd mutant retina (Figs. 3 to 5).

Rod nuclei were surrounded by a small amount of cytoplasm which contained a few small mitochondria. The terminals were small and usually contained a single synaptic ribbon in a given plane of section (Fig. 4). By contrast, cone nuclei were surrounded by a larger amount of cytoplasm with numerous large mitochondria. The terminals of the cone cells were larger than those of the rods, and they contained several synaptic ribbons and contacts on the basal surface (Fig. 5). These features distinguish rods and cones in the normal mouse retina.13 Of the 25 consecutive cells examined in random fields of posterior retina, 14 were cones and 11 were rods, almost exactly the expected proportion of each cell type based on our light microscope observations (Table I).

At 36 days of age, the only photoreceptor cells remaining had features characteristic of cone cells. This finding also confirmed our light microscope results. Portions of the inner segments projected beyond the external limiting membrane, but no outer segments were seen. The cells contained several synaptic ribbons and a small number of vesicles, and they still maintained contact with postsynaptic elements (Fig. 6).

Discussion

There has been speculation as to the identity of the last photoreceptor cells to survive in the rd/rd mouse retina. Sorsby et al.20 noted that all of the nuclei had several chromatin bodies when the outer nuclear layer was reduced to one row of nuclei. They interpreted this as a failure of rod cell differentiation. Another interpretation was given by Cohen20a and DiPaolo and Noell,21 who suggested that these cells might be cones. From our present observations, it is clear that the surviving cells are cones. Other previous morphological observations are consistent with our finding of rod cell degeneration preceding most of the cone cell degeneration in the rd/rd mouse retina. Blanks et al.22 and Cohen (personal communication in Blanks et al.22) have found that the alpha-type synaptic terminals (spherules) degenerate before the beta terminals (pedicles) in rd/rd mouse retinas, and the pedicles are, in fact, the synaptic terminals of cone cells in the mouse retina.13 In addition, our present electron microscope observations confirm those of Blanks et al. and of Cohen.

Mice with inherited retinal degeneration have been reported to show light-mediated behavioral responses by a number of workers2, 23–25 and to demonstrate a spectral sensitivity similar to that in normal mice, except with the threshold elevated by 5 log units.26 These studies were carried out long after the photoreceptor cells presumably had been lost from the retinas of the mice and the electroretinogram was extinguished.2, 22, 27 The previous investigators have gone to some lengths to explain how the animals might respond to light in the apparent absence of retinal photoreceptor cells. It is now clear that some photoreceptor cells survive, perhaps for the life of the animal. Karli et al.28 reported that some photoreceptor cells survive in rd/rd mice up to 1 year of age and that these cells make synaptic contact with cells of the inner plexiform layer. Cohen et al.29 have also seen some photoreceptor terminals in rd/rd mice at 90 days of age. We have extended this by showing that some cones survive up to at least 18 months of age. It would seem that despite the absence of outer segments, the surviving cones probably mediate the visually guided behavioral responses. Since rhodopsin is present in the outer plasma membrane of rod outer segments,30–33 it is possible that visual pigment is still present.
in the outer plasma membrane of the surviving cones in rd/rd mouse retinas and might provide a means of transducing the light signal.

Our findings of (1) a preferential survival of photoreceptor cells in the far peripheral retina, seen also at younger ages by Noell,27 and (2) the exclusive survival of cones, are consistent with the findings of electrophysiological studies on the superior colliculus of rd/rd mice. Dräger and Hubel34 have found that in 19-day-old rd/rd mice both visual responses and the retinotopic projection appear almost normal under photopic stimulus conditions. This is at an age when more than 98% of the rods have disappeared but most of the cones are still present (Table I). By day 24, Dräger and Hubel34 could no longer record visual receptive fields from the tectal regions subserving the central visual field, but within a peripheral ring the responses were still almost normal. In the ensuing 4 months, the peripheral responses faded, presumably as cones are progressively lost (Table I). Dräger and Hubel34 have also examined the tectal cells corresponding to the peripheral retina in 6- to 8-week-old rd/rd mice by measuring incremental thresholds while varying the background illumination. Their finding of an unresponsiveness to dim light is consistent with the virtual absence of rods at this age (Table I).

Since the complete loss of rod cells occurs while the majority of cone cells still exists, we tried to correlate the biochemical changes found by others with the morphological changes we observed in an attempt to gain insight into the biochemical composition of the cones. Unfortunately, it is impossible to draw firm conclusions about the biochemical nature of the surviving cones because of the low percentage of total retinal volume represented by the surviving cones (3%), the complexity of the surviving retinal tissue, possible transneuronal or adaptive changes in the surviving tissues, and other consid-
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Fig. 5. Higher magnification of the cone cell seen in the right side of Fig. 3. The nucleus of the cone cell is surrounded by a larger amount of cytoplasm than that of the rod (see Fig. 4). Some rough endoplasmic reticulum, polyribosomes, synaptic vesicles, and three synaptic ribbons (arrows) are present in the cone cell. dg, Degenerating (presumably rod) nucleus. (×12,000.)

erations (see Orr et al.35). Nevertheless, two observations are noteworthy and deserve further study. Orr et al.35 found a decrease in taurine concentration in the retina of rd/rd mice between postnatal days 30 and 90, which parallels the loss of cone cells that we find between these ages. In another study of rd/rd mice, Farber and Lolley36 report that after the early accumulation of cyclic guanosine monophosphate (GMP) and despite its decrease (correlated with loss of photoreceptor cells), the amount of cyclic GMP remains above normal. They suggest that this may be due to adaptive changes in cyclic GMP metabolism in the surviving retinal cells. It is not known whether the few surviving cones contain any of the excess GMP. Clearly, the presence and levels of cyclic GMP, as well as those of taurine and other substances, in the cones of the mouse retina await further resolution with techniques such as cytochemistry or immunocytochemistry.

Our understanding of the mechanism of gene action and the nature of the differential effect of the rd gene on the rods and cones in the mouse retina remains unclear. On the positive side, it is known that the action of the rd gene is intrinsic to the eye, based on (1) the in vitro degeneration of photoreceptors in whole eye cultures37,38 and (2) the presence of patches of photoreceptor degeneration in +/+ ↔ rd/rd experimental chimeras,39,40 both of which eliminate the need to consider circulating factors in the disease process. It is further known that the genetic defect is intrinsic to the neural retina, since in experimental chimeras made from pigmented rd/rd and albino +/+ (or vice versa) mice, a lack of correspondence is found between the distribution of mutant patches of pigment epithe-
Current thought on the mechanism of photoreceptor cell death in the rd/rd mouse is that a deficiency in cyclic nucleotide phosphodiesterase results in the accumulation of cyclic GMP which leads to the death of photoreceptor cells, a mechanism that has been simulated experimentally in developing eye rudiments of Xenopus embryos in vitro. Assuming that the rd gene affects only the photoreceptor cells, several possibilities exist which may explain the differential rate of degeneration in rod and cone cells. First, a product of the rd gene may affect the rod and cone cells differently. Second, the rd gene may be expressed in the two photoreceptor cell types at a different time. Third, the rd gene may be expressed only in rods, and cones may degenerate as a consequence of environmental changes. For example, the cone cells normally reside in the outer third of the outer nuclear layer and are separated from the vascular supply of the inner nuclear layer by several rows of rod nuclei. However, when the rod nuclei degenerate, the cone cells are closer to the vascular supply of the inner nuclear layer, and differences in oxygen tension or other features of the milieu probably exist. Other factors such as loss of outer segments may also lead to cell death. The precise mechanism by which rd gene leads to cone cell degeneration remains to be shown.

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REFERENCES


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