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Assessment of Retinal Degeneration in Outbred Albino Mice

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Evaluation of a pharmaceutical's safety includes assessment of the potential for ophthalmologic toxicity. These nonclinical studies commonly use various outbred stocks of mice. Pretest indirect ophthalmoscopic examinations in the commonly used outbred stock Hsd:ICR(CD-1) indicated that retinal degeneration was a problem in this particular outbred stock of mice. This prompted the authors to examine other stocks of outbred mice routinely used in the performance of nonclinical safety studies. Groups of mice were observed over a 13-week period to determine the progression and changing incidence of retinal degeneration. Light intensity in the room and caging was measured during the study, and it was determined that light did not play a direct role in the progression of the retinal degeneration observed during the study. Histomorphologic examination of the mouse eyes was performed at the end of the study to confirm the presence of retinal degeneration observed after ophthalmoscopic examination. The incidence of retinal atrophy in the various outbred stocks of mice was: Crl:CFW(SW)BR (98.3%), Tac(SW)fBR (80%), Tac:Icr:Ha(ICR)fBR (75%), Hsd:ICR(CD-1) (43.3%), and Crl:CF-1BR (3.0%). Retinal atrophy was not observed in the following outbred mice stocks: Crl:CD-1(ICR)BR, HsdWin:CFW1, and Hsd:NSA(CF-1). On the basis of these findings, it is highly recommended that pretest ophthalmologic screening be performed on mice to obviate pre-existing conditions from confounding or invalidating nonclinical study results.

The existence of an inherited retinal defect leading to rapid degeneration of photoreceptor cells has been known to develop in albino mice, as originally reported by Keeler (10) in 1924. This disorder was classified as being attributable to an autosomal recessive gene, and the gene symbol was noted as *rd* for retinal degeneration (3, 5). Other symbols have been assigned to this gene, and include *r*, *rd1*, and *Pdeb*^{rd1}. The *Pdeb*^{rd1} designation of the gene is based on identification of the actual subunit of the affected component of the photoreceptor cell (6). In mice that are found to be homozygous for the *rd* gene (*rd*/*rd*), retinal degeneration is observed to develop as a result of rod and cone photoreceptor cell death. The rods are seen to rapidly degenerate during the first three weeks after birth, and are determined to be completely absent by about seven weeks of age. Alternatively, the cones, which make up three percent of the photoreceptor cells in the mouse retina, appear to degenerate at a much slower rate and, indeed, some are found still to be present up to 18 months of age (1-3,13-15). A retinal degeneration slow (gene symbol *rds*) loci also has been reported. Mice that are determined homozygous for *rds*/*rds* gene are observed to have slower loss of the photoreceptor cells. This cell death has been observed to begin at approximately 14 to 21 days after birth, with complete absence of photoreceptor cells by one year of age. Heterozygous animals can manifest a slower rate of retinal degeneration that progresses throughout life (7, 9). The Jackson Laboratory has recently identified 16 naturally occurring mouse mutants that manifest photoreceptor degeneration, with preservation of other retinal cell types (4). Overview of the literature indicates that retinal degeneration can be caused by a variety of mutations in the photoreceptor cells.

Most of the investigations to date that have involved the study of retinal degeneration in mice have been conducted only in inbred strains of mice. However, there has been a report in the literature of investigations of retinal degeneration performed in outbred mice of ICR Swiss stock (10).

Outbred stocks of mice are commonly used in nonclinical safety studies. Complete assessment of a pharmaceutical agent's safety profile almost always includes evaluation of the potential of a compound to induce ophthalmic toxicity. Despite the genetic diversity that breeders attempt to maintain in outbred populations, alternative genetic constitutions develop over time that can lead to clinical conditions of which animal breeders and toxicologists are unaware. Retinal degeneration is one such confounding genetic condition, and retinal degeneration in albino mice is an important potential confounder in safety assessment studies. However, in addition to the genetic component of retinal degeneration, there is also the environmental aspect of retinal photoreceptor light sensitivity in mice. It has been reported that light intensity is a causative factor in retinal degeneration in pigmented and non-pigmented mice (15). While conducting drug safety studies in Hsd:ICR(CD-1) mice, which is an outbred stock of mouse commonly used in nonclinical safety studies, it was noted that a high percentage of these animals at a young age (four to five weeks) had retinal degeneration prior to initiation of exposure to a test article.

This finding prompted a more complete investigation of the phenomenon in this stock of mouse as well as other outbred stocks of mice commonly used in nonclinical safety studies. Light

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intensity was evaluated as to its potential to cause or exaggerate retinal degeneration in mice. Also, in an effort to evaluate the basic genetics of the retinal degeneration in the various outbred mice, an inbred strain of mouse (FVB/NCrlBR) was incorporated in the study as a positive-control group, since it has been previously established that this strain of mouse carries the *rd* gene. The C57BL/6J-Tyr$^{c\text{-}2J}$/+ mice were included as a negative-control group, since this inbred strain has been reported in the literature to be resistant to retinal degeneration (12). Determination of the actual mutation(s) causing the retinal degeneration observed in the outbred albino mice included in this study was not established. In light of this fact, the symbol rd will be used to indicate that a change is occurring in the photoreceptor cells that appears to have an inherited basis.

The ultimate goal of the study reported here was to investigate and report the incidence of retinal degeneration in outbred stocks of mice that are commonly used in safety assessment evaluations. The results will provide to investigators as well as regulators pertinent background information on the frequency with which retinal degeneration develops in these particular strains and stocks of mice. Such data will be useful in the analysis of the ophthalmic portions of study data. These results will also aid investigators in selecting which outbred stock of mouse to use for a given study, depending on the emphasis placed on the ophthalmologic evaluation included in the study.

Materials and Methods

Mice. Use of the following groups of mice to determine the incidence of retinal degeneration via indirect ophthalmoscopy and histomorphologic examination in the various stocks and strains was approved by the Institutional Animal Care and Use Committee. Groups were composed of the following types and numbers of mice. Thirty male and 30 female 4- to 5-week-old mice of each outbred stock were purchased from various vendors. The outbred stocks and their corresponding vendors were:

Swiss Webster (SW) background—Crl:CFW(SW) BR (Charles River Laboratories, Inc., Portage, Mich.); Tac:(SW)fBR (Taconic Farms, Germantown, N.Y.); and HsdWin:CFW1 (Harlan Sprague Dawley, Inc., Borchen, Germany).

Institute of Cancer Research (ICR) background—Crl:CD-1(ICR) BR (Charles River Laboratories, Inc., Raleigh, N.C.); Tac:Icr:Ha(ICR)fBR (Taconic Farms, Germantown, N.Y.); Hsd:ICR (CD-1) (Harlan Sprague Dawley, Inc., Frederick, Md.).

Non-Swiss albino background—Crl:CF-1 BR (Charles River Laboratories, Inc., Raleigh, N.C.), and Hsd:NSA(CF-1) (Harlan Sprague Dawley, Inc., Madison, Wis.).

Ten male and 10 female 4- to 5-week-old mice of each inbred strain were purchased. The FVB/NCrlBR strain was purchased from Charles River Laboratories, Inc., Raleigh, N.C., and the C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ strain was purchased from The Jackson Laboratories, Ann Harbor, Maine.

Health status. Vendor and in-house health surveillance reports indicated that the mice were free of the following viral, bacterial, mycoplasmal, and parasitic pathogens. On the basis of serologic test results, mice were determined to be free of Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, Theiler's mouse encephalomyelitis virus, reovirus type 3, epizootic diarrhea virus of infant mice, lactic dehydrogenase-elevating virus, mouse adenovirus, polyoma virus, mouse thymic virus, K virus, mouse cytomegalovirus, lymphocytic choriomeningitis virus, hantavirus, ectromelia virus, mouse parvovirus, *Encephalitozoon cuniculi*, *Mycoplasma pulmonis*, and cilia-associated respiratory bacillus. Bacteriologic culture was performed by the vendor on specimens from the respiratory and gastrointestinal tracts. The bacterial pathogens searched for included *Salmonella* spp., *Citrobacter freundii*, *Klebsiella* spp., *Steptococcus* spp., *Pasturella* spp., *Helicobacter* spp., *Streptobacillus moniliformis*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Bordetella bronchiseptica*, and *Mycoplasma* spp.

Results of the bacteriologic screening indicated that there were no relevant bacterial pathogens present in the animals, except in the male and female outbred Hsd:NSA(CF-1) animals, which were PCR test positive for *H. hepaticus.* However, there were no signs of clinical disease noted in the *H. hepaticus*-harboring animals. Parasite screening indicated that the mice were free of arthropods, helminths, coccidia, and *Giardia* spp.

Housing and husbandry. Mice were group housed in suspended, wire stainless steel cages and were individually identified by ear punch. Each group of mice was provided with sections of 1.5-inch-diameter PCV pipe, approximately four inches long, for purposes of environmental enrichment. Teklad Certified LM-485 Mouse/Rat Sterilizable Diet, 7012C (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) was provided ad libitum. Access to reverse osmosis-treated water was provided via an automatic watering system. Room lighting consisted of a 12/12-h light/dark cycle. The room was provided with approximately 20 changes of filtered 100% fresh air per hour. Room temperature and relative humidity were maintained at 20 ± 2°C and 54 ± 12%, respectively. Mice were observed a minimum of once each day for the purpose of assessing the general health of study animals. Animal care was in accordance to the 1996 NRC *Guide for the Care and Use of Laboratory Animals*.

Room light intensity measurements. Light intensity was measured (in foot candles [ft-candles]) in the room, at the front of the cage, and inside the cage, using a light meter (Model 93-1065F, Greenlee Textron, Rockford, Ill.). The average light intensity of the room was determined by subdividing the room into 12 individual equal-in-size sections. A central point in each section was then selected for placement of the measurement device at a height of one meter up from the floor. Subsection measurements were combined, and the average room light intensity was calculated. Room average light intensity was taken at the beginning of the study, midway through the study, and at the end of the study. The light intensity measurement inside the cage was taken by placing the light meter in the center of each cage, since this was considered to be the location that would provide the average light intensity that would most probably be experienced by the mice.

Ophthalmologic examination. With a few exceptions, ophthalmologic examination was performed on all mice at weekly intervals for 13 consecutive weeks, beginning when mice were four to five weeks old. The female Crl:CFW(SW) BR mice were not available until two weeks after the start of the study; therefore, these animals only received 11 ophthalmologic examinations, beginning when the animals were four weeks old. The Tac(SW)fBR and Tac:Icr:Ha(ICR)fBR stocks of mice were received shortly after the initial ophthalmologic examination was performed, subsequently undergoing only 12 examinations. Ophthalmologic examinations were performed by a board-certified veterinary ophthalmologist, using a Mentor indirect ophthalmo-

Table 1. Weekly incidence of retinal degeneration in male albino mice

Outbred or inbred albino mice	Weekly incidence of retinal degeneration (RD OU/n)					
	0[a]	1	2	3	4	5
FVB/NCrlBR n = 10	7/10 (70%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
Crl:CFW(SW)BR n = 30	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
Tac:(SW)fBR n = 30	NA	23/30 (76.7%)	23/30 (76.7%)	23/30 (76.7%)	23/30 (76.7%)	18/30 (60%)
Tac:Icr:Ha(ICR)fBR n = 30	NA	23/30 (76.7%)	23/30 (76.7%)	21/30 (70%)	23/30 (76.7%)	23/30 (76.7%)
Hsd:ICR(CD-1) n = 30	15/30 (50%)	17/30 (56.7%)	17/30 (56.7%)	18/30 (60%)	17/30 (56.7%)	17/30 (56.7%)
Crl:CF-1BR n = 30	1/30 (3.3%)	1/30 (3.3%)	3/30 (10%)	1/30 (3.3%)	1/30 (3.3%)	1/30 (3.3%)
Crl:CD-1(ICR)BR n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3.3%)
HsdWin:CFW1 n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
Hsd:NSA(CF-1) n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ n = 10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/9[b] (0%)	0/9 (0%)	0/9 (0%)

Table 1. Weekly incidence of retinal degeneration in male albino mice (cont.)

Outbred or inbred albino mice	Weekly incidence of retinal degeneration (RD OU/n)						
	6	7	8	9	10	11	12
FVB/NCrlBR n = 10	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
Crl:CFW(SW)BR n = 30	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
Tac:(SW)fBR n = 30	17/30 (56.7%)	22/30 (73.3%)	23/30 (76.7%)	24/30 (80%)	24/30 (80%)	23/30 (76.7%)	22/30 (73.3%)
Tac:Icr:Ha(ICR)fBR n = 30	24/30 (80%)	23/30 (76.7%)	23/30 (76.7%)	23/30 (76.7%)	23/30 (76.7%)	23/30 (76.7%)	23/30 (76.7%)
Hsd:ICR(CD-1) n = 30	17/30 (56.7%)	17/30 (56.7%)	17/30 (56.7%)	17/30 (56.7%)	17/30 (56.7%)	18/30 (60%)	17/30 (56.7%)
Crl:CF-1BR n = 30	1/30 (3.3%)	0/30 (0%)	1/30 (3.3%)	1/30 (3.3%)	0/30 (0%)	1/30 (3.3%)	1/30 (3.3%)
Crl:CD-1(ICR)BR n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
HsdWin:CFW1 n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
Hsd:NSA(CF-1) n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3.3%)	0/30 (0%)	0/30 (0%)
C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ n = 10	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)

[a]Mice were four to five weeks old at initial ophthalmologic examination.
[b]One male in moribund condition was euthanized.
RD OU = retinal degeneration, both eyes; NA = not available due to initial delay in receipt.

scope and a 60D-condensing lens. Pupils were dilated by instillation of the mydriatic agent, one percent tropicamide (Opticyl-1, Miza Pharmaceuticals USA, Inc., Fairton, N.J.). One drop per eye was applied approximately 30 to 60 min prior to examination. Additional amounts of dilating agent were instilled into the eyes if it was determined by indirect ophthalmoscopy that the degree of mydriasis was not adequate for examination.

Mice were manually restrained during the ophthalmologic examination. The technique for restraint involved the attending technician grasping the loose skin behind the ears and at the base of the skull with the thumb and index finger. The mouse's tail was held between the fourth and fifth fingers of the grasping hand. By grasping the skin at the base of the skull and behind the ears firmly, the eyes were slightly proptosed, which permitted the examining ophthalmologist to have an unobstructed view of the mouse's eye.

Histologic examination. To confirm that the clinical ophthalmologic findings of retinal degeneration correlated to actual histologic changes, such evaluation of the left eye from each mouse was performed. After the last ophthalmologic examination had been performed, the mice were euthanized by CO_2 asphyxiation and exsanguination by cardiocentesis. After asphyxiation and exsanguination, the head was removed to ensure death of the animal. Eyes were removed and placed in Davidson's solution. Orientation of the eyes to examine the retina by quadrants was not performed. Slides were prepared at Experimental Pathology Laboratories, Inc (EPL; Herndon, Va.), and were stained with hematoxylin and eosin. Histomorphologic evaluation was performed by a board-certified EPL pathologist.

Data interpretation. The incidence of retinal degeneration in most of the stocks and strains was determined over a 13-week period. Exceptions to this included the Tac(SW)fBR and the Tac:Icr:Ha(ICR)fBR stocks of mice because of a one-week delay in receipt, and the female Crl:CFW(SW)BR mice that had a two-week delay in receipt. The mice were evaluated by ophthalmologic examination at four to five weeks of age. The onset of retinal degeneration was compared over time, using the FVB/NCrlBR inbred strain of mouse as the positive-control group, since it carries the *rd* gene, and the C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ inbred strain of mouse as a negative-control group, since it is known to be resistant to retinal degeneration.

The effects of light intensity on retinal degeneration were also evaluated by comparing the incidence of retinal degeneration over time in relationship to the position of the mice in the cage rack. Analysis was performed by comparing animals in subsequent cage positions with the animals in the top caging level. The premise was based on findings that an increase in light intensity exposure increases the rate that light-sensitive animals will develop retinal degeneration. Accordingly, it was theorized that animals in the top cage level would develop retinal degeneration at a faster rate than animals in the lower cage levels as the light intensity within the cage would decrease as the cage level position moved away from the light source in the ceiling. The evaluation of the equality of means was made by use of one-way analysis of variance and the F-distribution to assess the degree of statistical significance. If statistically significant differences between means were found, the Dunnett's test was then used to determine the degree of significance. Systat, version 9.01 (SPSS, Inc., Chicago, Ill.) was used for the analysis.

Results

Ophthalmologic findings. Mice with retinal degeneration had severe attenuation or absence of retinal vessels and marked reduction in choroidal vascularity, resulting in bluish-white discoloration of the fundi. Examination of the fundi of mice with normal retinas revealed prominent retinal blood vessels and

Table 2. Weekly incidence of retinal degeneration in female mice

Outbred or inbred albino mice	Weekly incidence of retinal degeneration (RD OU/n)					
	0[b]	1	2	3	4	5
FVB/NCrlBR n = 10	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
Crl:CFW(SW)BR n = 30	NA	NA	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)
Tac:(SW) fBR n = 30	NA	25/30 (83.3%)	25/30 (83.3%)	24/30 (80%)	25/30 (83.3%)	25/30 (83.3%)
Tac:Icr:Ha(ICR)fBR n = 30	NA	25/30 (83.3%)	25/30 (83.3%)	20/30 (66.7%)	25/30 (83.3%)	26/30 (86.7%)
Hsd:ICR(CD-1) n = 30	7/30 (23.3%)	9/30 (30%)	9/30 (30%)	11/30 (36.7%)	9/30 (30%)	9/30 (30%)
Crl:CF-1BR n = 30	0/30 (0%)	0/30 (0%)	1/30 (3.3%)	0/30 (0%)	0/30 (0%)	1/30 (3.3%)
Crl:CD-1(ICR)BR n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
HsdWin: CFW1 n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
Hsd:NSA(CF-1) n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ n = 10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)

Table 2. Weekly incidence of retinal degeneration in female mice (cont.)

Outbred or inbred albino mice	Weekly incidence of retinal degeneration (RD OU/n)						
	6	7	8	9	10	11	12
FVB/NCrlBR n = 10	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
Crl:CFW(SW)BR n = 30	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)
Tac:(SW) fBR n = 30	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)
Tac:Icr:Ha(ICR)fBR n = 30	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)	25/30 (83.3%)
Hsd:ICR(CD-1) n = 30	9/30 (30%)	9/30 (30%)	9/30 (30%)	9/30 (30%)	10/30 (33.3%)	9/30 (30%)	9/30 (30%)
Crl:CF-1BR n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3.3%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
Crl:CD-1(ICR)BR n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
HsdWin: CFW1 n = 30	4/30 (13.3%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
Hsd:NSA(CF-1) n = 30	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)
C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ n = 10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)

See Table 1 for key.

choroidal flush. The incidence of retinal degeneration based on indirect ophthalmoscopic examination for the male and female inbred and outbred mice on a weekly basis is found in Tables 1 and 2. As expected, all of the FVB/NCrlBR mice of either sex were found to have retinal degeneration. The incidence of retinal degeneration was 100% in the male Crl:CFW(SW)BR and 96.7% in the females of this outbred stock. The Tac(SW)fBR and Tac:Icr:Ha(ICR)fBR mice also had a high incidence of retinal degeneration, with the male incidence at 73.3% and 76.7%, respectively, and the female incidence at 83.3% for both outbred stocks. The female Hsd:ICR(CD-1) mice had a 30% incidence of retinal degeneration, whereas that for males was almost double this value at 56.7%. The male Crl:CF-1BR mice were found to have a very low incidence of retinal degeneration (3.3%). The retinal degeneration-resistant mice, C57BL/6J-*Tyr*$^{c\text{-}2J}$/+, did not have any ophthalmologic evidence of retinal degeneration nor did the following stocks: female Crl:CF-1BR or male and female Crl:CD-1(ICR)BR, HsdWin:CFW1, and Hsd:NSA(CF-1).

Furthermore, review of these data revealed the presence of a baseline incidence of retinal degeneration, from which excursions in frequency of those affected occurred. Since severity of retinal degeneration in mice does not follow a course of waxing and waning over time, the most plausible explanation for these changes in frequency are the restraint technique itself. Quite often when mice are restrained a little too tightly to prevent biting, the retinas can become blanched in appearance, leading to misdiagnosis of retinal degeneration.

In addition to the presence of retinal degeneration, a few other incidental ocular findings were noted. One female HsdWin:CFW1, at four weeks of age, was observed to have a microphthalmic left eye with a cataract. Corneal opacities that would resolve over time were noted in various animals throughout the groups. One male and one female Crl:CR-1BR mouse were each observed to have a linear retinal scar in the right eye.

Light intensity values and effects. The average light intensity of the room during the study was determined to be 32.7 ft-candles at the beginning of the study, 31.4 ft-candles midway through the study, and 30.7 ft-candles at the end of the study. Since the room light intensity did not increase during the course of the study and cages were kept in the same position in the room throughout the study, the cage-side and inside-the-cage readings were only determined at the beginning of the study. The cage racks were made up of five rows of cages, with six cages across each row. Starting at the tops of the racks, the average light intensity at the cage face across the rows was: row 1 (top of rack), 28.6 ft-candles; row 2, 24.4 ft-candles; row 3, 20.7 ft-candles; row 4, 17.7 ft-candles, and row 5 (bottom of rack), 14.9 ft-candles. The average light intensity inside the cages across the rows, starting at the top of the racks, was: row 1, 1.23 ft-candles; row 2, 0.99 ft-candles; row 3, 0.87 ft-candles; row 4, 0.60 ft-candles, and row 5, 0.51 ft-candles.

Since the light source in the room was from overhead recessed fluorescent lights, the light intensity at the front of the cage as well as inside the cage was found to decrease from the top to the bottom of the cage rack, as might be expected. The actual light intensity to which the mice were consistently exposed was reflected by the values obtained inside the cages. These values were well below the magnitudes of the values (12.1 to 30.2 ft-candles) recommended for animals that are known to be susceptible to light-induced retinopathy (8, 11).

Statistically significant differences were not found for the inbred mice, when animals in the lower baskets were compared with animals in the upper baskets. However, a few significant differences were found in the incidence of retinal degeneration among the outbred mice, when animals in the lower baskets were compared with animals in the top basket.

Throughout the study, female Hsd:ICR(CD-1) mice in cages in rows 3 and 5 had a significantly ($P < 0.05$) lower incidence of retinal degeneration than did animals in row 1. Even though the incidence of retinal degeneration in the female Hsd:ICR(CD-1)

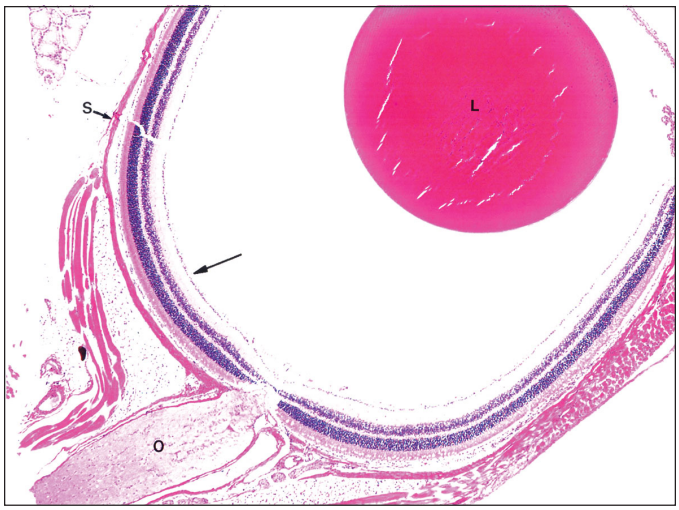

Figure 1. Photomicrograph of a section of the eye from a female Hsd:NSA (CF-1) mouse. Notice normal retina (arrow) with multiple layers (L = lens, O = optic nerve, S = sclera). H&E stain; magnification = 93×.

mice in rows 3 and 5 was significantly less, compared with that for mice in row 1, the finding was not considered to be biologically relevant. This conclusion is based on the fact that, if the decrease in retinal degeneration in these animals were truly the result of a decrease in exposure to light on the basis of the position in the rack, one would expect the mice in row 4 to also have a significant decrease in retinal degeneration, compared with that in animals in row 1. This was not the case, however, as the mice in row 4 did not have a significant decrease in the incidence of retinal degeneration, compared with that in mice in row 1. The incidence of retinal degeneration observed in this outbred stock of mice appeared to be sporadically distributed among individual animals and, apparently, did not progress over time, as might be expected if light exposure was a factor. Accordingly, the decrease in retinal degeneration in the mice in rows 3 and 5 was considered to be a random event.

Sporadically throughout the study, the following mice had a significantly decreased incidence of retinal degeneration, compared with the mice in row 1: male Tac(SW)fBR in row 4, week 6 ($P < 0.01$); male Tac:Icr:Ha(ICR)fBR in row 5, week 3 ($P < 0.01$); and female HsdWin:CFW1 in rows 2–5, week 6 ($P < 0.01$). The sporadic significant differences observed in the aforementioned outbred animals were attributed to the occasionally inaccurate ophthalmologic diagnosis of retinal degeneration in the mice due to the restraint technique rather than light exposure, since the incidence of retinal degeneration did not appear to progress over time.

Histopathologic findings. The retinal degeneration observed during ophthalmologic examinations performed throughout the study was found to be bilateral. Therefore, histomorphologic evaluation of the left eye only was performed on all animals at the end of the study to confirm the retinal degeneration observed via indirect ophthalmoscopy in the animals. Figure 1 shows the appearance of a normal retina. Figure 2 is a close-up of a normal retina, with the layers of retina labeled. Figure 3 and 4 depict the appearance of the atrophic retina that is typically associated with retinal degeneration. It should be noted that the diffusely atrophic retina is thinner than normal due to the diffuse absence of the photoreceptor layer (rods and cones), the outer nuclear layer, and the outer plexiform layer, as well as

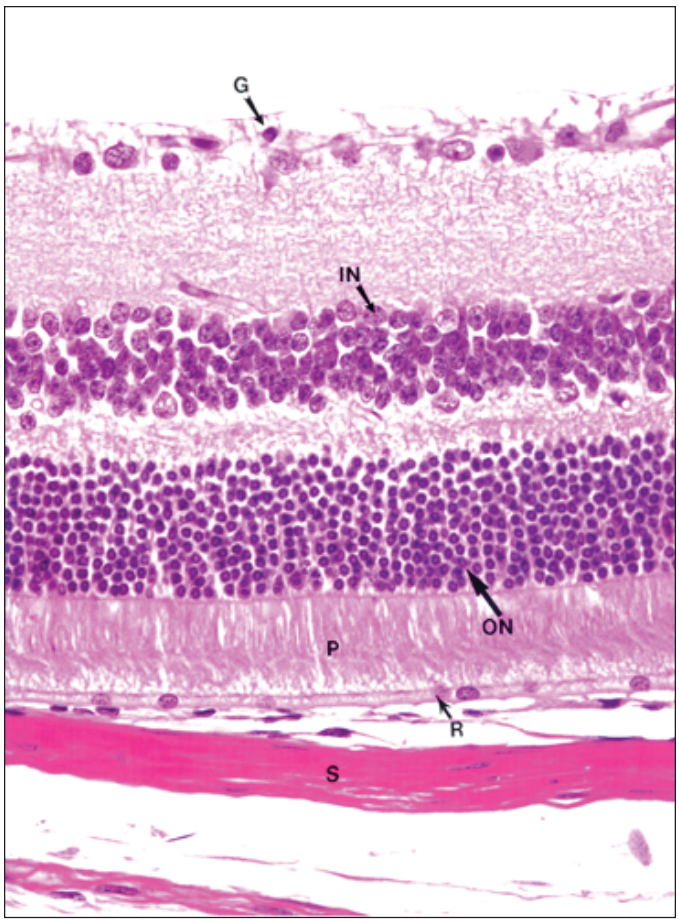

Figure 2. Photomicrograph of a section of normal retina from a male CRL:CD-1(ICR)BR mouse. Normal retinal layers include the ganglion cell layer (G), inner nuclear layer (IN) outer nuclear layer (ON), photoreceptor layer (rods and cones [P]), and retinal pigment epithelium (R). H&E stain; magnification = 917×. *See* Fig. 1 for key.

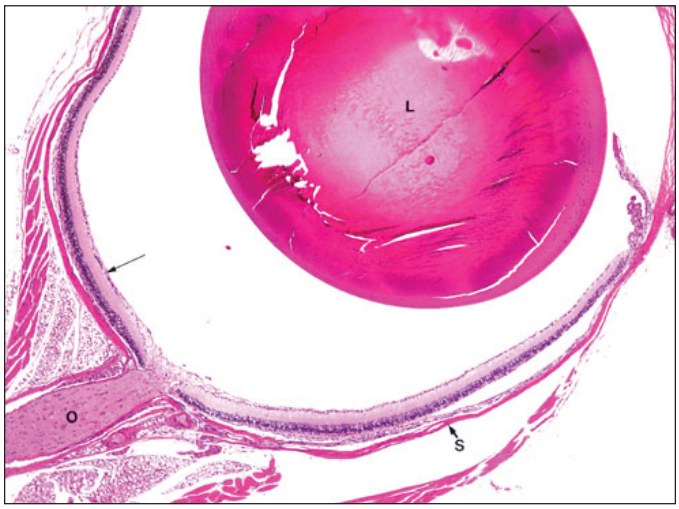

Figure 3. Photomicrograph of a section of the eye from a female CRL:CFW(SW)BR mouse. Notice diffusely atrophic retina (arrow) is thinner and has fewer layers than normal. H&E stain; magnification = 92×. *See* Fig. 1 for key.

so me focal thinning and minor nuclear loss and pyknosis of the inner nuclear layer. Because the outer layers were lost, the inner

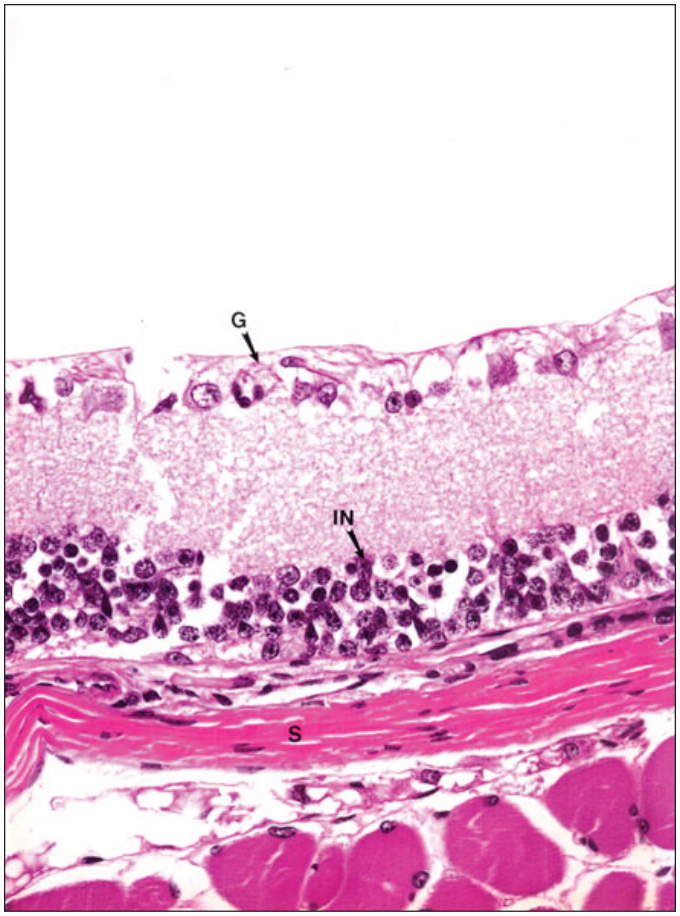

Figure 4. Photomicrograph of a section of retina from a male CRL:CFW (SW) BR mouse. Notice that the retina is diffusely atrophic and thinner than normal. Photoreceptor layers are absent. Inner nuclear layer (IN) is hypocellular, and exhibits nuclear shrinking and loss. The ganglion layer (G) appears unaffected. H&E stain; magnification = 911×. *See* Fig. 2 for key.

nuclear layer rested directly on the retinal pigmented epithelium. The retinal pigmented epithelial cells were usually slightly enlarged, and occasionally focally increased in number. The ganglion cell layer generally appeared unaffected. The underlying choroid and choroidal vessels were unremarkable in the eyes with atrophic retinas as well as in the eyes with normal retinas.

The diffuse retinal atrophy observed in these animals was considered moderate in severity, since this classification most appropriately described the extent of observed histomorphologic pathologic changes (outer layers lost, but inner layers preserved).

A few other minimal ocular findings were observed sporadically at low incidence in various mice. The findings included mononuclear cell infiltrates in the corneal limbus, choroid and iris, and lens cataract. Remnants of hyaloid vessels (attached to the optic disk or posterior portion of the lens) and retinal fold formations (in-foldings or rosette-like structures) were seen in a few animals, and were considered to be congenital anomalies of minor importance. Generally, minimal corneal neovascularization, epithelial vacuolization and hyperplasia, stromal pigmented macrophages, and/or basement membrane hypertrophy corresponded to the findings of grossly observed corneal opacities. Histomorphologic evaluation of the right eye was performed if gross findings, such as corneal opacity, were observed. Tables 3 and 4 contain summaries of the histopathologic findings.

Discussion

All animals survived to the end of the study, except for a C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ male that was euthanized during week 3 due to development of poor body condition, decreased activity, and an enlarged and domed cranium. Gross necropsy revealed a severely enlarged cranial cavity, exudation of serosanguineous fluid from the brain when the cranium was opened, and severe ventricular dilatation. These finding are consistent with gross diagnosis of severe hydrocephalus. Further histomorphologic evaluation was not conducted to definitively confirm this diagnosis. A male Tac:Icr:Ha(ICR)BR and a female C57BL/6J-*Tyr*$^{c\text{-}2J}$/+ were found dead on the last day of the study immediately prior to necropsy. The cause of death was not determined.

The incidence of retinal degeneration did not appear to increase over time for the majority of the inbred and outbred mice after the initial ophthalmologic examination at four to five weeks of age. This was not unexpected in the FVB/NCrlBR inbred mice since animals that are homozygous for the *rd* gene have generally developed retinal atrophy by four to five weeks of age (9). It is interesting to point out that, when comparing the outbred stocks that have a high percentage of retinal atrophy by four and five weeks of age (e.g. 100% of male Crl:CFW(SW)BR)

Table 3. Summary incidence table of ocular histopathologic findings in male mice

Histopathologic findings	FVB/ NCrlBR	Crl:CFW (SW)BR	Tac:(SW) fBR	Tac:Icr: Ha(ICR) fBR	Hsd:ICR (CD-1)	Crl:CF-1 BR	Crl:CD-1 (ICR)BR	HsdWin: CFW1	Hsd:NSA (CF-1)	C57BL/ 6J-*Tyr*$^{c\text{-}2J}$/+
Eye, left (No. examined)	(10)	(30)	(30)	(30)	(30)	(30)	(30)	(30)	(30)	(10)
Choroid, infiltrate, mononuclear cell	0	0	1	0	0	0	0	0	0	0
Cornea, basement membrane, hypertrophy	0	0	0	0	0	1	0	0	0	0
Cornea, epithelium, hyperplasia	0	0	0	0	0	1	0	0	0	0
Cornea, limbus, infiltrate, mononuclear cell	0	0	0	0	0	0	0	0	0	0
Cornea, neovascularization	0	0	0	0	0	2	0	0	0	0
Cornea, stroma, pigmented macrophages	0	0	0	0	0	1	0	0	0	0
Iris, infiltrate, mononuclear cell	0	0	0	0	0	0	0	0	0	0
Lens, cataract	0	0	0	0	0	0	0	0	0	0
Lens, persistent hyaloid vessel	0	0	0	0	1	0	0	0	0	0
Optic disk, persistent hyaloid vessel	0	0	0	0	0	0	0	0	0	0
Retina, atrophy	10	30	23	22	17	1	0	0	0	0
Retina, fold(s) formation	0	0	1	0	0	1	0	0	0	0
Eye, right (No. examined)	(0)	(0)	(0)	(0)	(0)	(2)	(1)	(0)	(0)	(0)
Cornea, epithelium, vacuolization	0	0	0	0	0	0	0	0	0	0
Cornea, limbus, infiltrate, mononuclear cell	0	0	0	0	0	0	1	0	0	0

Table 4. Summary incidence table of ocular histopathologic findings in female mice

Histopathologic findings	FVB/ NCrlBR	Crl:CFW (SW)BR	Tac:(SW) fBR	Tac:Icr: Ha(ICR) fBR	Hsd:ICR (CD-1)	Crl:CF-1 BR	Crl:CD-1 (ICR)BR	HsdWin: CFW1	Hsd:NSA (CF-1)	C57BL/ 6J-*Tyr*$^{c\text{-}2J}$/+
Eye, left (No. examined)	(10)	(30)	(30)	(30)	(30)	(30)	(30)	(30)	(30)	(10)
Choroid, infiltrate, mononuclear cell	0	1	0	0	0	0	0	0	0	0
Cornea, basement membrane, hypertrophy	0	0	0	0	0	0	0	0	0	0
Cornea, epithelium, hyperplasia	0	0	0	0	0	0	0	0	0	0
Cornea, limbus, infiltrate, mononuclear cell	0	0	0	0	0	1	0	1	0	0
Cornea, Neovascularization	0	0	0	0	0	0	0	0	0	0
Cornea, stroma, pigmented macrophages	0	0	0	0	0	0	0	0	0	0
Iris, infiltrate, mononuclear cell	0	0	0	0	0	0	0	1	0	0
Lens, cataract	0	0	0	0	0	0	0	1	0	0
Lens, persistent hyaloid vessel	0	0	0	0	0	0	0	0	0	0
Optic disk, persistent hyaloid vessel	0	1	0	0	0	0	0	0	0	0
Retina, atrophy	10	29	25	23	9	1	0	0	0	0
Retina, fold(s) formation	0	0	0	0	0	1	0	1	0	0
Eye, right (No. examined)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(1)
Cornea, epithelium, vacuolization	0	0	0	0	0	0	0	0	0	1
Cornea, limbus, infiltrate, mononuclear cell	0	0	0	0	0	0	0	0	0	0

with that observed in FVB/NCrlBR mice, it may be concluded that these outbred animals also may have been homozygous for an *rd* gene. This finding was not anticipated since the outbred animals would have been expected to have a smaller incidence of retinal degeneration. This position is based on the fact that these animals are supposed to be more genetically diverse, more heterozygous, and accordingly, more resistant to the development of retinal degeneration.

The findings from this study suggest that, in four of the outbred stocks [Crl:CFW(SW)BR, Tac(SW)fBR, Tac:Icr:Ha(ICR)fBR, and Hsd:ICR:(CD-1)], the breeding program ostensibly amplified the selection of an *rd* gene. Even though the incidence of retinal degeneration was not 100% in most of the aforementioned stocks, it was interesting that the incidence of heterozygotes that would be expected within an outbred population was not observed. This position is based on the observation that the incidence did not change after the mice reached five weeks of age. A possible explanation for this is that the animals were not observed for a sufficient period of time as it may take heterozygous animals months to years to develop retinal degeneration. If the study duration had been extended up to a year or longer, it is possible that more animals in the outbred stocks that currently did not exhibit retinal degeneration early may have eventually developed the condition.

It is noteworthy that, although male Crl:CFW(SW)BR mice had 100% incidence of retinal degeneration, female Crl:CFW(SW)BR mice had a 96.7% incidence. One female Crl:CFW(SW)BR appeared to be resistant to development of retinal atrophy; this mouse may have been truly resistant. Alternatively, since all the mice were albinos, it is possible that this female was not truly a Crl:CFW(SW)BR mouse. A similar scenario was observed in two Crl:CF-1BR mice (one male and one female) that each had retinal atrophy, whereas the remaining male and female Crl:CF-1BR animals did not have the condition. This finding either indicated that an *rd* gene was present in the population at an extremely low level, the retinal atrophy was caused by something other than an *rd* gene, or the mice were not true Crl:CF-1BR animals.

Overall, the incidence of retinal degeneration, as determined by ophthalmologic examination during the in-life portion of the study, correlated closely with the diffuse retinal atrophy found at the end of the study by histomorphologic evaluation of the eyes. Minor increases and decreases in incidence were seen in some of the outbred stocks during the course of the study, but these changes were judged to be iatrogenic in nature (i.e., the restraint technique). On the basis of the findings from this study, the indirect ophthalmoscopic examination of mice is an excellent tool to screen for the presence of retinal degeneration in a particular strain or stock of mouse.

On the basis of the results of histologic evaluation at the end of the study, significant differences in the incidence of retinal atrophy were observed in various strains and stocks of inbred/outbred mice. However, the severity of retinal degeneration was similar in all affected animals of all inbred strains and outbred stocks. Affected inbred/outbred mice did not exhibit sex-related differences, except for the Hsd:ICR(CD-1) mice, in which the incidence of retinal atrophy in males (17/30 [56.7%]) was almost twice as high as that in females (9/30 [30.0%]).

The largest incidence of retinal atrophy (both sexes combined) was found in the positive-control group of inbred FVB/NCrlBR mice (20/20 [100%]) and the outbred Crl:CFW (SW)BR mice [59/60 (98.3%)]. A high incidence of retinal atrophy was also present in the Tac:(SW)fBR [48/60 (80.0%)] and Tac:Icr:Ha(ICR)fBR [45/60 (75.0%)] stocks. The Hsd:ICR(CD-1) mice had an intermediate incidence of retinal atrophy [26/60 (43.3%)]. The Crl:CF-1BR mice had a very low level of incidence [2/60 (3.0%)] of retinal degeneration. Retinal atrophy was not observed in the following inbred and outbred mice: C57BL/6J-*Tyr*$^{c\text{-}2J}$/+, HsdWin:CFW1, Crl:CD-1(ICR)BR and Hsd:NSA(CF-1).

Investigators should be cognizant of inherent confounding conditions in the animals they might choose for use in safety studies. Such a condition would be the propensity of an animal to develop retinal degeneration. The presence of retinal degeneration may lead to the hopeless confounding of experimental data restricting interpretation and possible invalidation of the study. Furthermore, knowledge of such conditions prevents useless experimentation on animals. Our results presented here should aid researchers in making an informed choice when deciding which outbred stock of mouse will provide a suitable model for the data they are trying to obtain. Regulatory agencies also will benefit from the knowledge that retinal degeneration is an inherent condition in particular outbred stocks of mice and not a direct result of environmental conditions or treatment with a test article. Use of indirect ophthalmoscopy to determine whether mice have retinal degeneration prior to the start of a study is highly recommended and is considered to be an accurate in-life method of screening for this condition.

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