Slit-lamp Assessment of Onset of Cataracts in Black-eyed, Black-hooded Retinal Dystrophic Rats

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Various types of hereditary retinal degeneration have associated posterior subcapsular cataract (PSC). It has been claimed that in the Royal College of Surgeons (RCS) rat model of hereditary retinal dystrophy, the cataract is manifested unpredictably and does not display Mendelian inheritance. It was shown previously, however, that 100% of pink-eyed retinal dystrophic RCS rats had an onset of bilateral PSC at 7 to 8 weeks of postnatal age, and by 9 to 11 months, 23% of the animals had cataracts visible to the unaided eye. The congenic black-eyed retinal dystrophic RCS rat, however, is a better model for the generally more pigmented human eye. In the present work, it was found that 100% of black-eyed RCS rats had bilateral slit-lamp-detectable PSC beginning at 8 weeks of postnatal age, just as the pink-eyed rats did, despite the fact that dark-eye pigmentation is associated with a 10- to 35-day delay in the rate of degeneration in retinal areas other than the peripheral part of the inferior hemisphere. A higher incidence of mature cataracts in pink-eyed rats (23%) as compared with black-eyed rats (3%) suggests that the amount or intensity of light reaching the lens, retina, and pigmented epithelium may influence maturation of the cataract. However, if light is important in initiating the PSC, its influence was not decreased by dark pigmentation of the eye. RCS rats may be a model for an early onset type of human autosomal recessive retinal degeneration having a constant association of PSC. Invest Ophthalmol Vis Sci 24:654–657, 1983

Posterior subcapsular cataracts (PSC) are often seen in association with retinal degenerations, such as retinitis pigmentosa1 and gyrate atrophy of the retina and choroid.2 They also occur in the Royal College of Surgeons (RCS) rat model of autosomal recessive retinal dystrophy.3 The relation of PSC to retinal degeneration is not understood. Recently we demonstrated in pink-eyed, tan-hooded dystrophic RCS rats fed a natural ingredient NIH rodent diet4 that onset of PSC was between 7 and 8 weeks of age, at which time bilateral sugar grain-appearing opacities were distinguishable by slit-lamp examination.5 All rats examined at later ages had PSC, although only 23% of them developed mature cataracts by 9 to 12 months of age. This demonstrated that the PSC of the RCS rat occurred in a predictable fashion and at about the time when most of the rod photoreceptors had degenerated (about 2 months).

The congenic black-eyed retinal dystrophic RCS rat may be a better model for some type of hereditary retinal degeneration in humans, most of whom have pigmented eyes. The black-eyed rats were shown previously to have a much lower incidence of mature cataracts (3%) by 2.5 to 11 months of age,3 but the age of onset was not studied. Black-eye pigmentation

References
also has been shown to have a marked retarding effect on the rate of retinal degeneration, consisting of 10 days in the posterior retina and 35 days in the peripheral part of the superior hemisphere, but no effect in the peripheral part of the inferior hemisphere. These effects are the same as those produced by dark rearing of pink-eyed dystrophics. We applied the sensitive diagnostic slit-lamp technique to determine whether the black-eyed dystrophic had an early onset of cataractous change or whether occurrence of PSC was delayed in parallel with the delays in rates of retinal degeneration in the different retinal regions or the delay in onset of the mature cataracts.

This article describes our finding that slit-lamp-detectable PSC begins at the same time in the black-eyed as in the pink-eyed dystrophics, namely at 7 to 8 weeks of postnatal age. Eye pigmentation appears, therefore, not to delay onset of PSC. The RCS rat may have more direct relevance as a model for human PSC than for human retinal degeneration, since the specific human counterpart of the rat retinal disease has not been identified.

Materials and Methods. Diet: The diet used was a natural ingredient diet developed for conventionally reared laboratory mice and rats. All rats were fed this NIH diet and were progeny of rats similarly fed. The diet contained recommended levels of all known nutrients for rodents and has been used satisfactorily for many strains of rats at NIH. Animals: The rat strain used in the study was the black-eyed, black-hooded retinal dystrophic RCS/N\textsuperscript{rdy/rdy}−p\textsuperscript{*}. This strain is the same as the RCS-p\textsuperscript{*} strain of Dr. M. LaVail, and was introduced into the NIH(N) foundation colonies by cesarean sectioning in 1979.

Housing and lighting: Breeding pairs and young animals were housed in clear plastic cages with wire tops. Nonbreeding, older animals were housed in raised-wire stainless steel holding cages with solid metal covering. All cages were on racks under fluorescent-only lighting of maximum 500 lux intensity for 12 hrs, alternating with 12 hrs of darkness.

Observational methods: For observations in vivo, rats were anesthetized with ether and pupils were dilated with 1% tropicamide. Eyes were examined with either a handlight and unaided eye or with a Zeiss slit-lamp biomicroscope having camera attachment. Hydroxypropyl methylcellulose, 2.5% in isotonic NaCl solution (Goniosol), was applied to the cornea to prevent drying.

For observations in vitro, pupils were dilated in anesthetized rats and the rats were killed with ether or pentobarbital. Under a dissecting microscope, a cut was made with a scalpel blade parallel to the ora serrata and 1 to 2 mm posterior to it. Straight, angle-bladed microdissecting scissors were used to cut around the globe to free the posterior segment. The anterior segment was transferred into a plastic petri dish containing buffered saline solution to keep the lens afloat. The posterior aspect of the lens was examined at various angles under different illumination to view the cataract.

Results. Forty black-eyed retinal dystrophic RCS rats between the ages of 40 postnatal days and 6.5 months were examined by slit lamp (Table 1). In rats 40–48 days of postnatal age, no cataractous change was visible. By 53 days of age, however, all rats examined had bilateral posterior subcapsular cataracts (Fig. 1); some of the PSC were milder than the one depicted.

The cataracts were also observed in vitro, as described in Methods. In rats 9 to 10 weeks of postnatal age, the cataractous saucer-shaped plaque was seen to occupy a position displaced anterior to the capsule rather than immediately adjacent to it.

Of 25 dystrophic black-eyed rats aged 6.5 months, all had bilateral slit-lamp-detectable PSC, but none had developed mature cataracts visible to the unaided eye (Table 1).

Discussion. Previous investigators concluded that the RCS rat cataract, unlike the autosomal recessive retinal dystrophy, is manifested unpredictably, and not inherited in a Mendelian fashion. Our studies, however, have shown that early cataractous changes in dystrophic RCS rats, regardless of eye pigmentation, occur in as constant and predictable a way as the retinal degeneration. This does not indicate whether the cataract is a direct or indirect conse-
Fig. 1. Mild sugar-grain type posterior subcapsular cataract of pigmented retinal dystrophic RCS rats visible by slit-lamp biomicroscopy. Slit-lamp photograph (×18). These cataracts were seen in both lenses of all rats examined over the age of 8 postnatal weeks.

sequence of the function of the rdy gene, which is expressed as a phagocytic defect in the retinal pigmented epithelium. A possible direct relationship to the function of the rdy gene cannot be explored since the products of the normal and mutant alleles are presently unknown. However, a testable hypothesis is that PSC, which accompany retinal degenerations of diverse origins, may be initiated by some pathologic aspect or chemical product of degenerating retina.

If some quantitative relationship exists between retinal degeneration, toxic products and initiation of cataract, then the death of a similar number of rod photoreceptor cells would be expected to be required in pink- and black-eyed rats for a cataract to begin. When the slit-lamp-detectable PSC appear at about 55 days, the retinal degeneration has proceeded to a loss of approximately 75% of the thickness of the outer nuclear layer in the black-eyed and a loss of about 88% in the pink-eyed dystrophic rat in the posterior retina. In the pink-eyed rat most of the rod photoreceptor nuclei have degenerated by about 66 days, while in the black-eyed rat a similar degree of degeneration is not reached until about 100 days; in peripheral areas (other than inferior) the delay is greater. More rigorous quantitation of the comparative number of photoreceptor cells lost by 8 weeks in pink- and black-eyed dystrophics would require subtraction of the amount of DNA remaining in these retinas from the normal DNA/retina value in the corresponding congenic pink- or black-eyed RCS control (RCS-ryy"−p/p or RCS-ryy"p") at the same age. Yates et al. studied DNA/mg wet wt of retina in strains of pink- and black-eyed dystrophic rats closely related to the present ones. DNA values for pink-eyed Campbell dystrophies were compared with normal Wistar albino and those for black-eyed Hunter dystrophies with the normal Piebald Virol Glaxo rats used to develop the Hunter strain. At 8 weeks of age, DNA values in the pink- and black-eyed dystrophics differed from corresponding normal values by similar amounts. However, the amounts of DNA/retina, which would have enabled estimation of the numbers of photoreceptors dying by 8 weeks of age, were not given.

Because of these known marked differences in the rates of progress of retinal degeneration in the black- and pink-eyed dystrophic RCS rats, we expected that a delay of onset of PSC would occur if some quantitative factor related to the degeneration was initiating the pathology. Instead, the identical age of onset of PSC in the two strains appears at first glance to mean that black pigmentation of the iris and retinal pigmented epithelium, which greatly reduces the entrance of light and its scattering within the eye, does not affect PSC initiation in the dramatic way it affects the rate of retinal degeneration. The observation that dark rearing of pink-eyed dystrophics and black-eye pigmentation have the same retarding effect on the rate of retinal degeneration may suggest that onset of PSC would also occur at 7 to 8 weeks in the pink-eyed RCS rats reared in darkness and that light is not necessary for initiation of PSC. Light may be involved in the maturation process, however, because the incidence of cataracts visible to the unaided eye is much greater in the pink-eyed (23%) than in the black-eyed dystrophic (3%).

Our observation on excised 9- to 10-week-old lenses revealed a clear area between the capsule and the saucer-shaped PSC. This clear zone suggests that the cataract develops within a short period of vulnerability at about 7 to 8 weeks of age in both pink- and black-eyed rats rather than during a prolonged and indefinite period. After the initial damage has occurred, the next lens fiber cells appear to remain healthy, as might be expected if essentially all the toxic material from degenerating cells had been exported. Studies are in progress to show the light microscopic histopathologic lesion at its onset and its subsequent change in location or composition.
The RCS rat has been thought to provide a model for some type of autosomal recessive retinal degeneration with early onset. It may have more immediate relevance for human PSC than for human retinal degeneration, because the specific human counterpart of the rat disease is unknown. If the pink- and black-eyed models have validity for human PSC, it seems worthwhile to explore whether the degree of pigmentation of the human iris and retinal pigmented epithelium may influence maturation of the cataracts and whether shielding from bright light could have a protective effect.

**Key words:** RCS rats, retinal diseases, animal disease models, cataracts

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