

Notes

Congenetic strains of RCS rats with inherited retinal dystrophy

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INHERITED RETINAL DYSTROPHY in the rat, first discovered in 1938 by Bourne *et al.*³, is a recessively inherited disorder that results in the progressive loss of photoreceptor cells beginning in the third postnatal week, i.e., during late stages of retinal development. Similarities between the rat disease and retinitis pigmentosa in man were pointed out in the initial report³, and the animals still are called "retinitis pigmentosa rats" by some workers.

Since the discovery of the mutation, there have been numerous biochemical and morphological studies of the diseased retinas (see references in LaVail and Battelle¹). One of the most important findings has been that the rate of photoreceptor degeneration is slowed considerably when the pink-eyed dystrophic rats are reared in the dark⁶. This observation of environmental modification of the genetic disorder has led, in part, to the experimental therapeutic measure of light deprivation for patients with early stages of retinitis pigmentosa¹.

A general problem with this and all other studies has been that the presumed normal control animals in every case have differed from the dystrophic rats at many genetic loci in addition to the one responsible for

the retinal dystrophy itself. For example, the data interpreted as an increase in rhodopsin content of the dystrophic retinas⁶ might, in fact, have reflected a decrease in the "controls". Likewise, there have been melanin pigmentation differences in many studies. This might be a particularly important variable with reference to the effect of environmental light on the pace of the retinal disease^{11,17}. It is also pertinent with reference to light deprivation as a possible therapeutic measure for retinitis pigmentosa in humans, since most of the dystrophic rat eyes, in contrast to human eyes, have been almost without melanin pigment. The solution to this problem is to prepare congenic strains of rats differing at the retinal dystrophy locus or at some other locus of choice. In this note we describe the development and some characteristics of strains of rats congenic with the inbred pink-eyed RCS (Royal College of Surgeons) strain but which differ in melanin pigmentation.

Methods

The early history and pigmentation genotype of the RCS strain have been described by Sidman and Pearlstein¹⁸. These tan-hooded, pink-eyed animals are homozygous for the recessive retinal dystrophy gene and carry the following pigmentation genes: *ala B/B C/C D/D h/h p/p* (see Robinson¹⁴ and Searle¹⁵ for further characterization of these pigmentation genotypes and phenotypes). The genotypes were recognized when partially inbred RCS animals were mated in 1962 to noninbred albinos (CR rat stock, Charles River Breeding Laboratories, North Wilmington, Mass.) and produced all black-eyed, black-hooded F₁ progeny. F₂ rats were produced, and for the next 3 years black-eyed (*p/+*) animals were maintained by a series of intercrosses, crosses with cousins, and crosses with partially inbred RCS stock. In 1965, when the RCS strain reached inbred status (F₂₀), one of the black-eyed animals with retinal dystrophy was mated to an RCS rat and produced a segregating litter of pink-eyed (*p/p*) and black-eyed (*p/+*) animals, all with retinal dystrophy. Later, black-eyed progeny were repeatedly backcrossed to RCS rats; these animals have now reached the N₁₁ backcross generation. The strain was considered to have reached congenic status at the N₇ generation, and following the rules recommended for nomenclature of inbred strains of rats⁷, we designate this strain RCS-*p/+*. The strain will continue to be maintained by repeated backcrosses of black-hooded rats (*p/+*) to RCS; this mating gives litters with approximately equal numbers of black-eyed (*p/+*) and pink-eyed (*p/p*) animals, all with retinal dystrophy.

Two of the black-hooded N₇ rats were intercrossed and produced four litters of N₇, F₁ progeny, of which about 25 percent were albinos and about 25 percent were pink-eyed. The two N₇ (*p/+*) parents, therefore, still carried the closely-linked albinism gene, *c*, and were of the genotype *c +/+ p*, the *c +* linkage group having come from the original CR rat of 1962. Several of the N₇, F₁ albinos were progeny-tested for status at the *p* locus by crossing with RCS. A male and female littermate were classified as *c +/c +* on the basis of the finding that all progeny in two litters (15 or more animals) were black-

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hooded. These albinos were then intercrossed to produce N_7 , F_2 albino progeny, which were subsequently bred brother \times sister and are presently at the N_7 , F_6 generation. We designate this congenic strain RCS-c. In order to maintain the congenic status, the animals will be backcrossed to RCS every 5-7 generations and then intercrossed again to retrieve and maintain the albino line.

The rats have been maintained in a 12-hour light-12-hour dark environment at room illumination of approximately 20-35 ft-c from overhead fluorescent lamps. The rooms were temperature-controlled at $23 \pm 1^\circ\text{C}$, and the animals were fed Charles River Original Rat-Mouse Formula *ad libitum*.

Results and Discussion

The effect of eye pigmentation on the rate of retinal dystrophy in these congenic lines is presented elsewhere in detail¹¹. In brief, the black eye pigment in RCS-*p/+* rats slows the progression of the disease to the same extent as dark-rearing of pink-eyed RCS rats. In the posterior retina, photoreceptor degeneration is slowed by about 10 days. In the far peripheral retina, the disease is slowed by about 30-35 days along the horizontal meridian and in the superior half of the eye. No slowing of the disease occurs in the inferior half of the eye along the vertical meridian. The small amount of eye pigment in pink-eyed RCS rats apparently is inadequate to slow the disease, since no differences in morphology or rhodopsin content were seen in albino RCS-c versus pink-eyed RCS rat retinas.

Rats with retinal dystrophy were originally described as also having cataracts highly variable in incidence, time of onset and maturation^{2,4}. About 40 percent of the animals never showed signs of cataracts. Of those with cataracts, most showed some lens changes by the middle of the third month of age. Sometimes the cataract developed rapidly in one eye and progressed slowly or remained stationary in the other. Some cataracts never reached maturity. We have found mature cataracts in the eyes of rats in each of our strains (Table I). A large proportion of animals appeared to have unilateral cataracts; however, since no ophthalmoscopic examination was made, we cannot be sure that the grossly normal lens was, in fact, completely normal. We have probably underestimated the overall incidence, as we may have missed very small opacities.

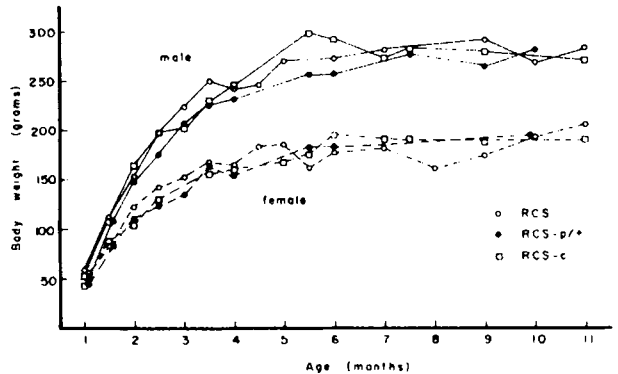


FIGURE 1—Growth curves for pink-eyed RCS, black-eyed RCS-*p/+* and albino RCS-*c* rats. Each point is the mean of 3-8 animals.

All of our lines have a low incidence of microphthalmia, as judged by gross examination (Table I). About the same percentage of additional animals have a slight narrowing of the aperture between the eyelids. We routinely discard these animals.

The growth curves for the RCS and congenic strains are illustrated in Figure 1. The animals in the different strains develop in body size at virtually the same rate. Body weights plateau at about 275 gms in males and 185 gms in females at 5-5.5 months of age. The extent of melanin pigmentation has no effect on general growth.

We did not measure the average life expectancy of RCS rats. We deliberately maintain a relatively young colony, since older animals appear to be more susceptible to respiratory ailments. We have maintained a few pink-eyed and black-eyed animals to 27 months of age.

The mean litter size is similar in the RCS and two congenic strains. Based on 32-37 litters from each line, and counting only the first four litters from any one dam, the mean litter sizes (\pm S.E.M.) were 6.76 ± 0.13 for RCS, 7.13 ± 0.37 for RCS-c and 7.38 ± 0.34 for RCS-*p/+*, values statistically indistinguishable from one another. Inbreeding may be a significant factor in this relatively small litter size, but in the original description of retinal dystrophy in a noninbred stock, the animals

Table I. Incidence of mature cataracts and microphthalmia*

Strain	No. examined	No. with cataract		% cataract	No. with microphthalmia		% microphthalmic
		Unilateral	Bilateral		Unilateral	Bilateral	
RCS	131	24	7	23.7	4	1	3.8
RCS- <i>p/+</i> †	70	2	0	2.9	2	0	2.9
RCS-c	66	4	4	12.1	3	0	4.5

* Based on gross examination of rats 2.5-11 months of age

† Only black-eyed animals were examined

were characterized as reproducing only "reasonably well"².

Yates *et al.*¹⁷ have recently described the development of a partially inbred line of pigmented rats with retinal dystrophy (Hunter strain). The source of their animals was the pink-eyed, tan-hooded Campbell stock which, like the RCS strain, derives from the original dystrophic animals described by Bourne *et al.*³. Yates *et al.*¹⁷ found that degeneration in the posterior retina is slowed by about 7 days, a finding very similar to our observations in the black-eyed RCS-*p/+* rats¹¹. Body weights are about the same in the Campbell, RCS, and congenic RCS-*p/+* rats, but the Hunter rats continue to grow with advancing age, perhaps a reflection of their genetic heterogeneity. No cataracts were found in the 34 black-eyed Hunter rats that were examined¹⁷, whereas RCS and the two congenic strains have a low incidence (Table I). We would anticipate that for some studies, particularly immunological and biochemical investigations, the use of congenic strains differing at one or a few pigmentation loci would be preferable to comparing black-eyed Hunter and pink-eyed Campbell animals.

We and other investigators have outcrossed rats with retinal dystrophy to other inbred and noninbred stocks to produce hybrid animals of different pigmentation types^{8,13,17}. In no instance were animals reported with modified rates of retinal dystrophy as has been described in outcrossed mice with inherited retinal degeneration (see references in Keeler⁹). The only modifying factor observed thus far appears to be essentially nongenetic and relates directly to the effective retinal illumination, modulated either by varying the ambient illumination^{6,13}, or the eye pigmentation^{11,17}. Furthermore, the rate of progression of the disease does not appear to have been modified by inbreeding programs^{3,6,11,13,17}.

We anticipate interest in the black-eyed RCS-*p/+* rats by investigators in vision research for several reasons. Further studies are needed to determine whether retinal dystrophy in the rat has relevance to any of the forms of human retinitis pigmentosa. Since the human eye is pigmented in most cases, the black-eyed RCS-*p/+* rat would seem to be a better model than the pink-eyed rat. Several electrophysiological procedures are easier to perform on pigmented eyes than on albino or pink eyes, and the c-wave of the electroretinogram is even said to be missing in albino rat eyes⁵. The congenic animals may be useful also for studying the combination of retinal dystrophy and 1) possible behavioral effects of pigmentation genes¹⁰, and 2) the reduced ipsilateral central projection of retinal ganglion cells in albino rats¹².

As mentioned above, it would be useful to have congenic strains differing only at the retinal dystrophy and closely linked loci, in addition to the presently described animals that all have retinal dystrophy but differ at pigmentation loci. We are developing such congenic strains differing from RCS rats at the retinal dystrophy locus. These should serve as the proper controls for the dystrophic animals, and a description will be forthcoming when the stocks have reached congenic status.

Summary

Two congenic strains of RCS rats, RCS-*p/+* and RCS-*c*, have been developed that differ from the parental strain at genetic loci affecting pigmentation. Inbred RCS rats are pink-eyed, while RCS-*p/+* rats produce segregating litters of pink-eyed (*p/p*) and black-eyed (*p/+*) offspring, and RCS-*c* rats are albinos. All the strains are homozygous for the mutant form of the retinal dystrophy gene. The black eye pigment in RCS-*p/+* rats slows the progression of the retinal degeneration by about 10 days in the posterior retina and by about 30–35 days in the peripheral retina in the superior half of the eye. No slowing of the disease occurs in the inferior half of the eye along the vertical meridian. All the strains are similar in body weight and litter size, and show a low incidence of cataract and microphthalmia.

Literature Cited

1. BERSON, E.L. Light deprivation for early retinitis pigmentosa. A hypothesis. *Arch. Ophthalmol.* 85:521–529. 1971.
2. BOURNE, M.C., D.A. CAMPBELL, and M. PYKE. Cataract associated with an hereditary retinal lesion in rats. *Brit. J. Ophthalmol.* 22:608–613. 1938.
3. ———, ———, and K. TANSLEY. Hereditary degeneration of the rat retina. *Brit. J. Ophthalmol.* 22:613–623. 1938.
4. ——— and H. GRÜNEBERG. Degeneration of the retina and cataract; a new recessive gene in the rat (*Rattus norvegicus*). *J. Hered.* 30:130–136. 1939.
5. DODT, E. and K. ECHE. Dark and light adaptation in pigmented and white rat as measured by electroretinogram threshold. *J. Neurophysiol.* 24:427–445. 1961.
6. DOWLING, J.E. and R.L. SIDMAN. Inherited retinal dystrophy in the rat. *J. Cell Biol.* 14:73–109. 1962.
7. FESTING, M. and J. STAATS. Standardized nomenclature for inbred strains of rats. Fourth listing. *Transplantation* 16:221–245. 1973.
8. HERRON, W.L., Jr., B.W. RIEGEL, E. BRENNAN, and M.L. RUBIN. Retinal dystrophy in the pigmented rat. *Investig. Ophthalmol.* 13:87–94. 1974.
9. KEELER, C.E. Reoccurrence of four-row rodless mice. *Arch. Ophthalmol.* 84:499–504. 1970.
10. ——— and H.D. KING. Multiple effects of coat color genes in the Norway rat, with special reference to temperament and domestication. *J. Comp. Psychol.* 34:241–250. 1942.
11. LAVAIL, M.M. and B.-A. BATTLE. Influence of eye pigmentation and light deprivation on inherited retinal dystrophy in the rat. *Exp. Eye Res.* in press. 1975.
12. LUND, R.D. Uncrossed visual pathways of hooded and albino rats. *Science* 149:1506–1507. 1965.
13. NOELL, W.K. Aspects of experimental and hereditary retinal degeneration. In: *Biochemistry of the Retina*. C.N. Graymore, Ed. Academic Press, London. p. 51–72. 1965.
14. ROBINSON, R. *Genetics of the Norway Rat*. Pergamon Press, Oxford. 1965.
15. SEARLE, A.G. *Comparative Genetics of Coat Colour in Mammals*. Logos/Academic Press, London/New York. 1968.
16. SIDMAN, R.L. and R. PEARLSTEIN. Pink-eyed dilution (*p*) gene in rodents: increased pigmentation in tissue culture. *Dev. Biol.* 12:93–116. 1965.
17. YATES, C.M., A.J. DEWAR, H. WILSON, A.K. WINTERBURN, and H.W. READING. Histological and biochemical studies on the retina of a new strain of dystrophic rat. *Exp. Eye Res.* 18:119–133. 1974.