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Development and degeneration of retina in *rds* mutant mice: observations in chimaeras of heterozygous mutant and normal genotype

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SUMMARY

In homozygous *rds* mutant mice the photoreceptor cells lack outer segment discs and slowly degenerate. In the heterozygotes the receptor cells develop abnormal outer segments and show altered disc shedding properties as revealed by the pigment epithelial phagosome content. The receptor cells also degenerate at a slower rate than in the homozygotes. The nature of the interaction resulting in dilution of the retinal lesion in the heterozygous retina was analysed in a series of chimaeras consisting of *rds*/+ and +/+ genotypes, which also differed in colour genes.

In 64 % of the chimaeras (18 out of 28) presence of both *rds*/+ and +/+ types of photoreceptors could be detected by electron microscopy. The relative proportion and patch size of the two components varied greatly between individuals but the location of the two types of photoreceptors was not related to the genotypes of the overlying pigment epithelial cells. Frequent occurrence of abnormally large phagosomes, resembling the *rds*/+ phenotype, was noted regularly in both *rds*/+ and +/+ types of pigment epithelial cells located above *rds*/+ types of receptors, but not in the cells of either genotype located above normal receptors. In the eyes examined at 12–18 months, localized and partial depletion of the perikaryal population in the outer nuclear layer was observed, and the location of such areas was also unrelated to the genotypes of the pigment epithelial cells. These findings confirm that the *rds* gene acts within the neural retina and possibly within the receptor cells and further show that the genetic interaction between the *rds* gene and its normal allele in the retina of the heterozygous mice takes place within the receptor cells.

INTRODUCTION

In mice homozygous for the *rds* (retinal degeneration slow) gene (Van Nie, Ivanyi & Demant, 1978) the photoreceptor cells fail to develop outer segment discs (Sanyal & Jansen, 1981; Cohen, 1983; Jansen & Sanyal, 1984) and slowly degenerate (Sanyal, De Ruiter & Hawkins, 1980). In the heterozygous individuals the photoreceptor cells develop morphologically abnormal outer segments, show an abnormal pattern of disc shedding and also degenerate, though degeneration starts later than in the homozygotes and progresses at a much slower rate (Hawkins, Jansen & Sanyal, 1985).

Analyses of retinal changes in chimaeric mice, consisting of *rds*/*rds* and +/+ cells have demonstrated autonomous expression of both genotypes, suggesting

Key words: chimaera, mouse, retina, *rds* gene, outer segments, disc shedding, degeneration.

that the *rds* gene acts within the neural retina and most likely within the photoreceptor cells (Sanyal & Zeilmaker, 1984). In order to understand the nature of genetic interaction leading to the dilution of the lesion in the retina of the heterozygous mice, we have undertaken light and electron microscopic studies on the retina of experimental chimaeras consisting of *rds*/+ and +/+ genotypes at different ages. The results show that (1) chimaeric distribution occurs frequently, (2) variable proportions of heterozygous mutant and normal cells are seen among the individual chimaeras and (3) their phenotypic expression, including altered disc shedding property, is independent of surrounding tissues, suggesting that co-dominant expression of the *rds* gene and its normal allele takes place within the photoreceptor cells.

MATERIALS AND METHODS

Two series of chimaeric mice were produced by combining 8-cell embryos of heterozygous *rds*/+ and normal +/+ genotypes. In one series embryos obtained from crossing between albino 020/A*rds* mice and normal albino Balb/cHeA mice were combined with those from pure strain, pigmented C3Hrd$^+$ mice. The latter strain is a congenic line of C3Hf/HeArd in which the *rd* gene has been substituted by the normal allele (Sanyal & Bal, 1973); it is also homozygous for the normal allele of the *rds* gene. In another series, embryos of pigmented C3H*rds*/+ genotype, obtained by crossing between C3H*rds*, a congenic line of C3Hrd$^+$ mice in which the *rds* gene has been introduced from the 020/A*rds* strain, and the homozygous normal C3Hrd$^+$ mice were combined with embryos from pure strain normal albino Balb/c mice.

For the production of chimaeric mice the procedures outlined by Mintz (1971) and Tarkowski (1964) were used. Normal adult females were allowed to mate and females with a vaginal plug were killed at the appropriate time. Two 8-cell-stage embryos were denuded of zona pellucida in 0·5 % pronase and were aggregated with the aid of a micromanipulator (Leitz) in a drop of culture medium under oil and incubated *in vitro*. The next day early chimaeric blastocysts were transferred to the uterine horns of recipient females on day 2 of pseudopregnancy (plug = day 0), which were then allowed to develop to term. After weaning, the animals were maintained in standard conditions with normal, cyclic 12 h light (06.30–18.30 h) and 12 h dark (18.30–06.30 h) periods.

One eye from each of the chimaeras was surgically removed at 2 months postnatal and was examined by light and electron microscopy. All fixations were performed between 15.00 and 16.00 h. The eyes were fixed in mixtures of aldehydes and osmium tetroxide, and embedded in low viscosity epoxy resin (Spurr, 1969) as described earlier (Sanyal & Jansen, 1981). Prior to embedding, each eye was cut into four quadrants and 1·0 μm thick radially cut sections from these quadrants were stained in toluidine blue for light microscopy and phagosome counting. To count phagosomes the procedures described by LaVail, Blanks & Mullen (1982) were followed. 37·5 μm-long stretches of albino pigment epithelium located over stretches of exclusively normal (+/+) or over stretches of exclusively *rds*/+ receptor outer segments were marked with an ocular micrometer and used as unit sample. Large phagosomes having a diameter range between 0·75 μm and 2·2 μm, which is about half of the width of the pigment epithelial cell soma of 4·4–4·5 μm in the central retina, and very large phagosomes that are greater than 2·2 μm across were counted. Three different samples were used from each eye. For the convenience of comparison mean values and standard errors of mean were calculated and expressed as counts per 100 μm (Table 2).

Ultrathin sections, taken from selected regions, were contrasted in alcoholic uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Philips EM 300 electron microscope. The other eye of the chimaeras was examined when the individuals were between 12 and 18 months old. In most cases the eyes were fixed in Orth's fluid and embedded in glycol methacrylate and serial sections were made at 3 μm. The sections were stained by the PAS procedure with

haematoxylin as counterstain as described earlier (Sanyal *et al.* 1980). A few of these later stage eyes were also examined in the electron microscope as above.

RESULTS

A total of 28 individuals were born from aggregated embryos and survived the period of experiment (Table 1). Of these individuals, 18 (64%) showed the presence of both *rds*/+ and normal +/+ types of receptors in the neural retina and their pigment epithelium also contained albino and pigmented cells. One individual was chimaeric in the pigment epithelium, with predominantly +/+ type of cells, but the neural retina appeared to contain exclusively +/+ type cells. All of the 18 individuals were also chimaeric in the coat with variable proportions of parental genotypes. The rest of the animals, as judged by the phenotypic distribution in the coat, pigment epithelium and the neural retina, were exclusively composed of one of the parental genotypes.

Ultrastructural observations on the chimaeric retina

In the individuals showing a chimaeric distribution in the neural retina, the presence of receptor cells from the two genotypes could be easily identified by the morphological differences in their outer segments (Fig. 1). In the receptor layer +/+ areas were seen as typically consisting of elongated elements with orderly piles of disc structures. In contrast, adjacent *rds*/+ areas consisted of large, irregular whorls of concentric layers of membranes. The phenotypic distinctions of these areas were sharp and they resembled those of the parental strains in all respects. Both types of receptor cells were often seen to be located under +/+ (Fig. 1) as well as under *rds*/+ (Fig. 2) types of pigment epithelial cells. The genotype of the overlying pigment epithelial cells could be identified by the presence or absence of melanin. In all of the individuals showing chimaeric distribution in the neural retina the pigment epithelium was also chimaeric but there was no spatial relationship in the cellular distribution between the two layers. Although no quantitative analysis was undertaken in this study, based on visual comparison of the electron micrographs, a considerable variation in the relative proportions of the cells derived from the two donor genotypes was recorded among the chimaeras. While, in some of the eyes, samples of the

Table 1. *Frequency of chimaeric (↔) or unigenotypic* (rds/+ *or* +/+) *distribution in pigment epithelium (PE)/neural retina (NR) in two series of chimaeric mice*

Series	Donor genotypes	No. of mice	PE / NR	+/+ / +/+	↔ / +/+	↔ / ↔	↔ / *rds*/+	*rds*/+ / *rds*/+
I	F$_1$*rds*/+ *c*/*c** C3H +/+ *C*/*C*	15		1	1	8	0	5
II	C3H*rds*/+ *C*/*C* Balb/c +/+ *c*/*c*	13		1	0	10	0	2

* F$_1$ hybrid between 020/A*rds*/*rds* and Balb/c +/+.

receptor layer examined showed a preponderance of *rds*/+ type of outer segments with a few of the +/+ type in between, in a few other chimaeras relatively more +/+ type outer segments were present. Within the sections of the chimaeric eyes

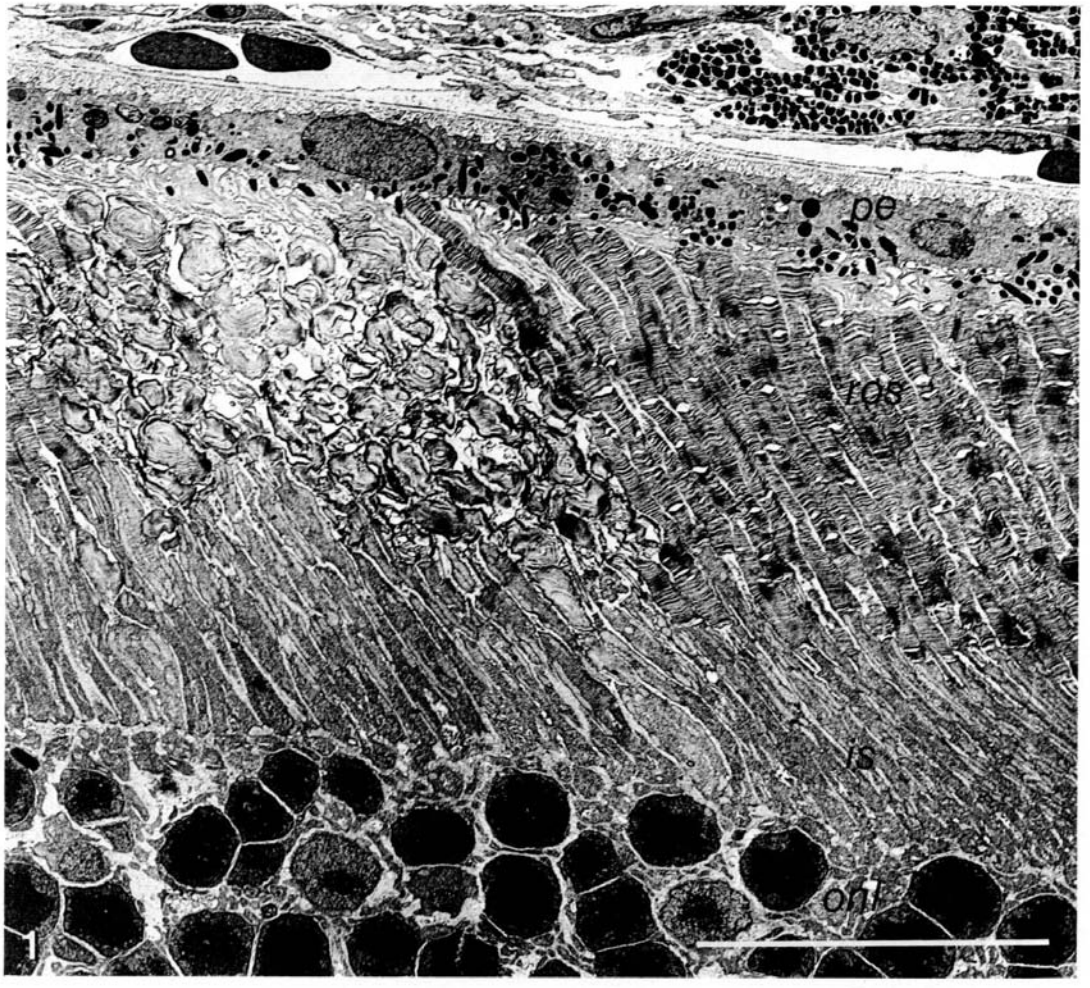
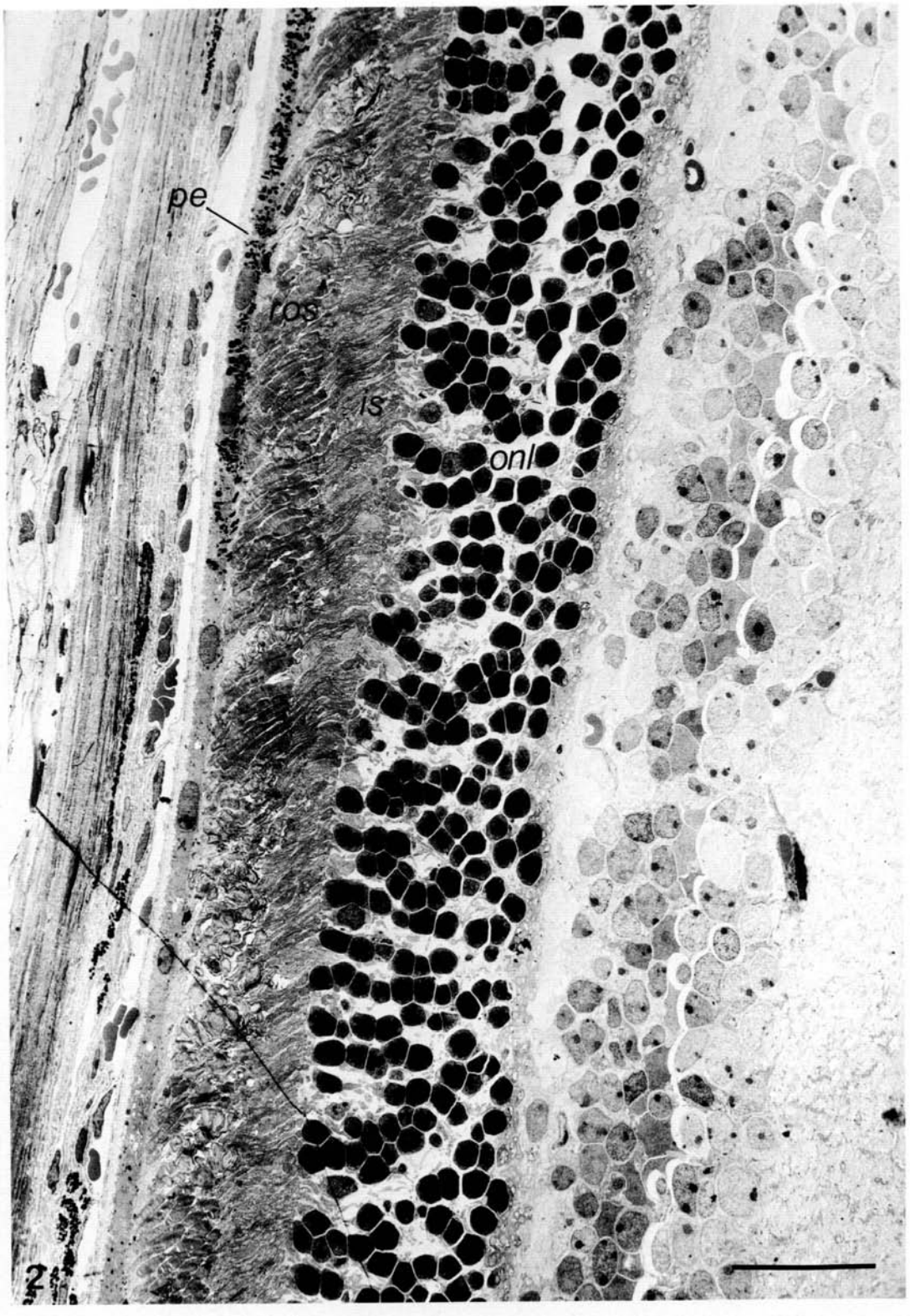


Fig. 1. Electron micrograph of outer retina from a 2-month-old chimaeric mouse containing albino *rds*/+ and pigmented normal (+/+) genotypes. Normal outer segments marked by elongated and regularly arranged piles of disc structures are located next to an area of outer segments containing irregular whorls which are typical of the heterozygous *rds*/+ retina. The cells in the overlying retinal pigment epithelium over both types of outer segments contain melanin (dark, round or oval bodies) and are, therefore, of normal (+/+) genotype. *pe*, pigment epithelium; *ros*, receptor outer segments; *is*, inner segments; *onl*, outer nuclear layer. Bar, 20 μm.

Fig. 2. Low magnification electron micrograph showing a wider area of retina from an albino *rds*/+ ↔ pigmented +/+ chimaeric mouse. Under pigment epithelial cells (*pe*) of both genotypes, receptor outer segments (*ros*) showing the characteristic heterozygous mutant phenotype are seen interspersed between stretches of normal outer segments. Note that the distribution of the two types of outer segments is patchy and the patches are of variable sizes. *is*, inner segments; *onl*, outer nuclear layer. Bar, 20 μm.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showing a more balanced distribution, the two types of receptors were located in patches of highly variable sizes (Fig. 2). In the neural retina profiles of a few, or even a single, outer segment(s), resembling the *rds*/+ phenotype could be found among completely normal-appearing outer segments. In the same way, normal outer segments were often surrounded by *rds*/+ type receptors.

Disc shedding of rds/+ *and normal outer segments in the chimaeric retina*

In the chimaeric eyes large phagosomes were more frequently observed in the pigment epithelial cells located over the *rds*/+ type of outer segments than in the pigment epithelial cells located over normal (+/+) type of outer segments (Fig. 3). In order to understand the relationship between the receptor genotype and the pigment epithelial cell genotype in controlling the disc shedding function, the phagosome counts in the albino stretches of the pigment epithelium overlying the two types of receptors were compared quantitatively. It should be noted that the albino cells in one series of chimaeras were genotypically *rds*/+, whereas in the other series the albino cells were of +/+ genotype. As a result of the patchy distribution and overlap of the two components within and between the two retinal layers, sufficient lengths of albino pigment epithelium overlying either of the two receptor types were not so frequently encountered. However, in three of the chimaeras from each of the two series appropriate samples were present. Data obtained from these individuals (Table 2) showed clearly that the number of phagosomes was high in the pigment epithelial cells of both genotypes when they were located over *rds*/+ receptors, thus resembling the *rds*/+ phenotype in the unigenotypic animals (Hawkins *et al.* 1985). In the pigment epithelial cells of both genotypes located over normal outer segments, the phagosome count was low and close to the figures obtained in unigenotypic animals with normal retina (Hawkins *et al.* 1985). Thus, phagosome content of the pigment epithelial cells was not related to their own genotype but to that of the underlying receptor cells.

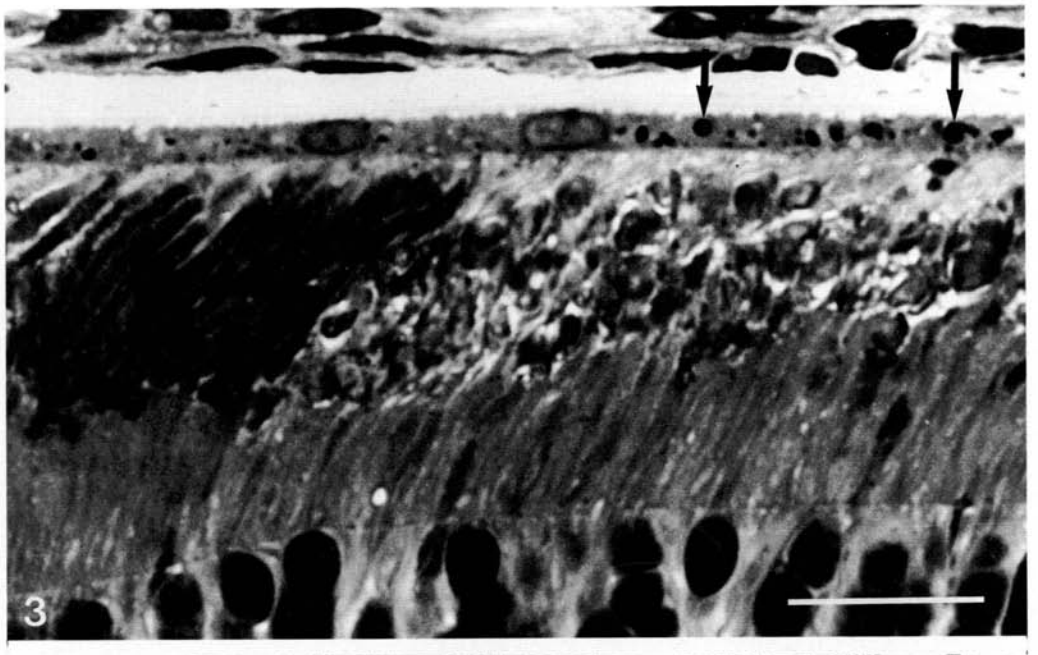
Photoreceptor cell degeneration in chimaeric retina

In the retina of the heterozygous mutant *rds*/+ mice degeneration of the photoreceptor cells started from around the age of two months and resulted in the

Fig. 3. Light micrograph of outer retina from a 2-month-old chimaeric mouse containing albino *rds*/+ and pigmented +/+ genotypes; toluidine blue staining. The overlying pigment epithelium is albino and therefore of *rds*/+ genotype. Note that large phagosomes (arrow) are present only in the region overlying the *rds*/+ type receptor cells but not over the normal receptor cells. Bar, 20 μm.

Fig. 4. Light micrograph of retina from an 18-month-old chimaeric mouse of pigmented *rds*/+ and albino +/+ composition; PASchiff staining. Note localized reduction of the outer nuclear layer (arrow); the outer segments located above are also irregular in appearance. Bar, 20 μm.

Fig. 5. Retina from another 18-month-old chimaera; PASchiff staining. Right-hand side of the photograph shows normal outer segments and normal thickness and proportion of the outer nuclear layer; the retinal region on the left-hand side shows depletion and reduction of the outer nuclei and irregular appearance of the outer segments. Bar, 20 μm.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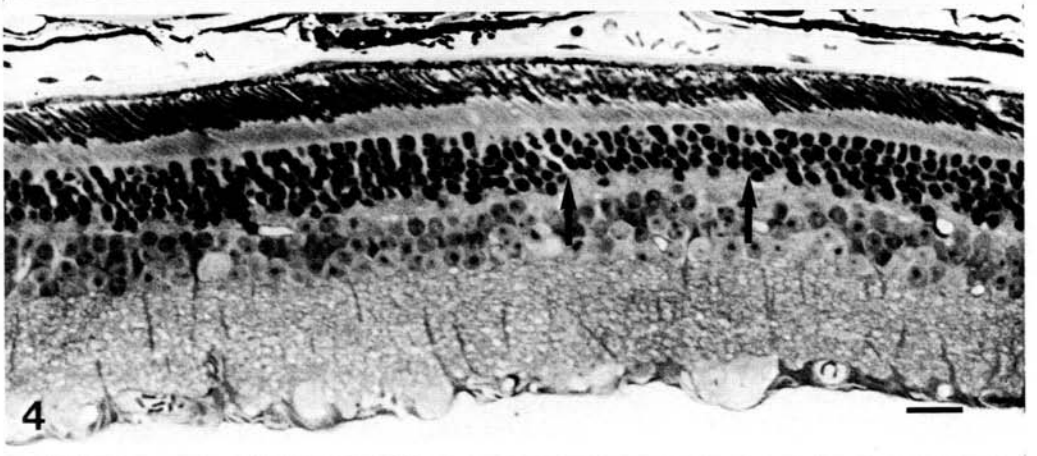
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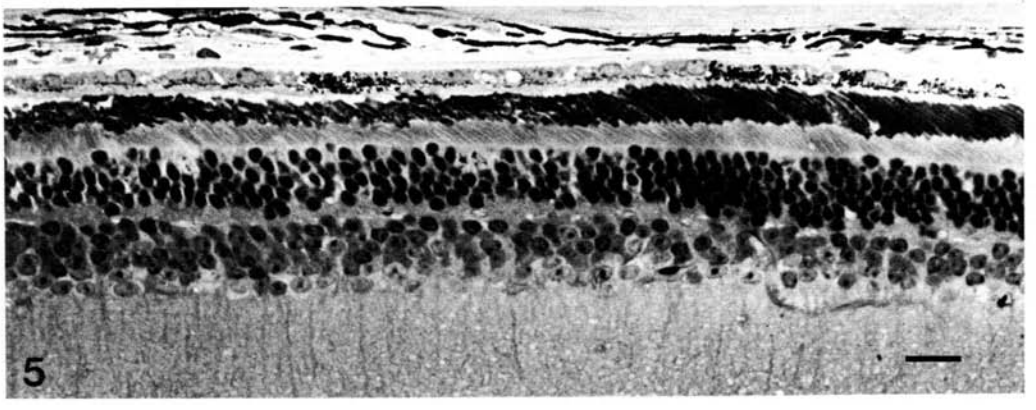
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Table 2. *Frequency of large (diam. > 0·75 μm) and very large (diam. > 2·2 μm) phagosomes in the albino cells of the pigment epithelium (PE) overlying normal (+/+) or heterozygous mutant* (rds/+) *photoreceptors (see Fig. 3) in two series of chimaeric mice*

	PE	+/+ c/c			
	NR	+/+		*rds*/+	
Donor genotypes		Large	Very large	Large	Very large
+/+ c/c		2·4 ± 0·8	0·0 ± 0·0	19·6 ± 0·9	3·9 ± 2·1
rds/+ C/C		2·7 ± 0·5	0·0 ± 0·0	18·7 ± 2·9	4·7 ± 1·1
		2·1 ± 0·8	0·0 ± 0·0	13·9 ± 2·4	5·9 ± 0·3
	(mean)	2·4 ± 0·7	0·0 ± 0·0	17·4 ± 2·1	4·8 ± 1·2
	PE	*rds*/+ c/c			
	NR	+/+		*rds*/+	
Donor genotypes		Large	Very large	Large	Very large
+/+ C/C		3·0 ± 0·6	0·0 ± 0·0	12·7 ± 3·9	5·6 ± 0·3
rds/+ c/c		2·4 ± 0·3	0·0 ± 0·0	15·1 ± 0·9	2·4 ± 0·8
		2·7 ± 0·0	0·9 ± 0·0	16·0 ± 1·9	5·0 ± 1·8
	(mean)	2·7 ± 0·3	0·3 ± 0·0	14·6 ± 2·3	4·3 ± 1·0
Control		+/+		*rds*/+	
		Large	Very large	Large	Very large
	(mean)	1·6 ± 0·1	0·3 ± 0·1	13·4 ± 1·6	3·0 ± 0·8

Each line represents one chimaeric individual; numbers are means of three samples with standard error of mean, expressed as counts per 100 μm-long stretch of PE.

reduction of the outer nuclear layer. The rate of degeneration was very slow so that reduction of the outer nuclear layer was clearly discernible only at the relatively late age of 9–12 months, and at around 18 months the outer nuclear layer was reduced to about half of its original thickness. Complete loss of all receptor cells, as observed in the homozygous mutants around the age of 9–12 months, was never recorded in the heterozygotes (Hawkins *et al.* 1985).

The eyes of chimaeric mice, examined at 12–18 months, showed partial loss of receptor cells resulting in localized reduction of the thickness of the outer nuclear layer or in some areas localized thinning of the perikaryal population (Figs 4, 5). However, such regions with depleted outer nuclei were located under both normal and *rds*/+ types of pigment epithelial cells. It should be kept in mind that loss of discrete *rds*/+ cells in a region overwhelmingly populated by normal cells might not be recognizable. Patches showing localized and partial depletion of outer nuclei also showed considerable variation in size.

DISCUSSION

The technique of experimental chimaeras has often proved useful in the analysis of sites of gene expression in the retina of rodents. In *rd*/*rd* ↔ +/+ chimaeric

mice lack of correlation of cell distribution in the neural retina and the pigment epithelium has been interpreted as autonomous expression of the *rd* gene within the neural retina (Mintz & Sanyal, 1970; LaVail & Mullen, 1976; Sanyal & Zeilmaker, 1976). In contrast, positive correlation between the distribution of mutant cells in the pigment epithelium and the site of photoreceptor cell degeneration in the retina of *rdy/rdy*↔+/+ chimaeric rat has provided clear evidence that the *rdy* gene acts primarily within the pigment epithelial cells of the mutant rat (Mullen & LaVail, 1976). In a recent study on chimaeric mice containing homozygous *rds* mutant and +/+ genotypes it has been shown that the distribution of the mutant photoreceptor cells is independent of the pigment epithelial cell genotype and, furthermore, the expression of the mutant phenotype is complete and independent of the surrounding +/+ cells (Sanyal & Zeilmaker, 1984). Analysis of chimaeric retina of *rds/rds*↔*rd/rd* mice in the same study has further provided strong indication, though not conclusive evidence, that the *rds* gene acts intracellularly within the photoreceptor cells.

In the retina of *rds*/+↔+/+ chimaeras the full spectrum of phenotypic changes that characterize the heterozygous mutant retina, e.g. abnormal outer segments, altered pattern of disc shedding and slow death of the photoreceptor cells, has been recorded just as in the retina of unigenotypic heterozygous mice. Chimaeric distribution in the neural retina has been noted frequently. The frequency of occurrence is about the same as for the chimaeric distribution in the retinal pigment epithelium and in the coat colour. The relative proportions of the two genotypes appeared to be variable among the chimaeric individuals and the spatial distribution of the two types of receptor cells has been found to be unrelated to the cell distribution in the pigment epithelium. Thus, the present findings confirm and reinforce the earlier conclusion that the *rds* gene acts within the photoreceptor cells and, furthermore, show that the interaction between the *rds* gene and its normal allele leading to the dilution of the retinal lesion in the heterozygous mice results from codominant expression of the genes within the receptor cells.

Apart from the structural differences, the *rds*/+ and +/+ receptor cells in the chimaeric retina also maintain their characteristic differences in disc shedding property. In the retina of vertebrates the distal tips of the rod outer segments are shed and incorporated in the retinal pigment epithelium as phagosomes (Young & Bok, 1969; Spitznas & Hogan, 1970). The turnover of phagosomes in the pigment epithelium is a measure of the rate of disc shedding and shows a cyclic daily change (LaVail, 1976; Basinger, Hoffman & Matthes, 1976). In the normal retina of mice the highest frequency of the phagosomes is recorded around the beginning of the light hours and drops to the lowest level around the end of the light hours (Besharse & Hollyfield, 1979). In contrast, phagosome counts in the pigment epithelium of *rds*/+ mice have shown an abnormally high frequency around the end of the light hours and many of these phagosomes have been found to be larger than normal (Hawkins *et al.* 1985). In the present study the phagosome profile of the pigment epithelial cells has been found to be independent of the pigment

epithelial cell genotype and appears to be related to the underlying receptor cell type. This observation clearly shows that the pigment epithelium does not play a causal role in the abnormal disc shedding pattern of the *rds*/+ receptor cells. More significantly, however, this observation shows the importance of intracellular factors in the control of the disc shedding pattern of the photoreceptor cells.

The pattern of turnover of phagosomes over a 24 h period follows a circadian rhythm that is entrained by the daily change of light but remains unaffected if darkness is prolonged (LaVail, 1980). Whereas prolonged exposure to light inhibits disc shedding (Goldman, Teirstein & O'Brien, 1980; Teirstein, Goldman & O'Brien, 1980), a period of darkness initiates disc shedding (Hollyfield, Besharse & Rayborn, 1976). It has been shown in frogs that the two eyes of an individual may be made to show different and independent patterns of disc shedding by experimentally altering the light exposure of single eyes (Hollyfield & Basinger, 1978). The different patterns of disc shedding by *rds*/+ and +/+ receptor cells in the chimaeric retina of individual eyes further show that some of the factors controlling disc shedding operate independently inside the photoreceptor cells.

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REFERENCES

BASINGER, S., HOFFMAN, R. & MATTHES, M. T. (1976). Photoreceptor shedding is initiated by light in the frog retina. *Science* **194**, 1074–1076.

BESHARSE, J. C. & HOLLYFIELD, J. G. (1979). Turnover of mouse photoreceptor outer segments in constant light and darkness. *Invest. Ophthal. Vis. Sci.* **18**, 1019–1024.

COHEN, A. I. (1983). Some cytological and initial biochemical observations on photoreceptors in retinas of *rds* mice. *Invest. Ophthal. Vis. Sci.* **24**, 832–843.

GOLDMAN, A. I., TEIRSTEIN, P. S. & O'BRIEN, P. J. (1980). The role of ambient lighting in circadian disc shedding in the rod outer segment of the rat retina. *Invest. Ophthal. Vis. Sci.* **19**, 1257–1267.

HAWKINS, R. K., JANSEN, H. G. & SANYAL, S. (1985). Development and degeneration of retina in *rds* mutant mice: Photoreceptor abnormalities in the heterozygotes. *Expl Eye Res.* **41**, 701–720.

HOLLYFIELD, J. G. & BASINGER, S. F. (1978). Photoreceptor shedding can be initiated within the eye. *Nature, Lond.* **274**, 794–796.

HOLLYFIELD, J. G., BESHARSE, J. C. & RAYBORN, M. E. (1976). The effect of light on the quantity of phagosomes in the pigment epithelium. *Expl Eye Res.* **23**, 623–635.

JANSEN, H. G. & SANYAL, S. (1984). Development and degeneration of retina in *rds* mutant mice: Electron microscopy. *J. comp. Neurol.* **224**, 71–84.

LAVAIL, M. M. (1976). Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science* **194**, 1071–1074.

LAVAIL, M. M. (1980). Circadian nature of rod outer segment disc shedding in the rat. *Invest. Ophthal. Vis. Sci.* **19**, 407–411.

LAVAIL, M. M., BLANKS, J. C. & MULLEN, R. J. (1982). Retinal degeneration in the *pcd* cerebellar mutant mice. 1. Light microscopic and autoradiographic analysis. *J. comp. Neurol.* **212**, 217–230.

LAVAIL, M. M. & MULLEN, R. J. (1976). Role of the pigment epithelium in inherited retinal degeneration analyzed with experimental mouse chimaeras. *Expl Eye Res.* **23**, 227–245.

MINTZ, B. (1971). Allophenic mice of multiembryo origin. In *Methods in Mammalian Embryology* (ed. J. C. Daniel, Jr), pp. 186–214. San Francisco: W. H. Freeman & Co.

MINTZ, B. & SANYAL, S. (1970). Clonal origin of the mouse visual retina mapped from genetically mosaic eyes. *Genetics* **64 (Suppl.)**, 43–44.

MULLEN, R. J. & LAVAIL, M. M. (1976). Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimaeras. *Science* **192**, 799–801.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

SANYAL, S. & BAL, A. K. (1973). Comparative light and electron microscopic study of retinal histogenesis in normal and *rd* mutant mice. *Z. Anat. EntwGesch.* **142**, 219–238.

SANYAL, S. & JANSEN, H. G. (1981). Absence of receptor outer segments in the retina of *rds* mutant mice. *Neurosci. Lett.* **21**, 23–26.

SANYAL, S., DE RUITER, A. & HAWKINS, R. K. (1980). Development and degeneration of retina in *rds* mutant mice: Light microscopy. *J. comp. Neurol.* **194**, 193–207.

SANYAL, S. & ZEILMAKER, G. H. (1976). Comparitive analysis of cell distribution in the pigment epithelium and the visual cell layer of chimaeric mice. *J. Embryol. exp. Morph.* **36**, 425–430.

SANYAL, S. & ZEILMAKER, G. H. (1984). Development and degeneration of retina in *rds* mutant mice: Light and electron microscopic observations in experimental chimaeras. *Expl Eye Res.* **39**, 231–246.

SPITZNAS, M. & HOGAN, M. J. (1970). Outer segments of photoreceptors and the retinal pigment epithelium. *Archs Ophthal.* **84**, 810–819.

SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**, 31–43.

TARKOWSKI, A. K. (1964). Patterns of pigmentation in experimentally produced mouse chimaerae. *J. Embryol. exp. Morph.* **12**, 106–108.

TEIRSTEIN, P. S., GOLDMAN, A. I. & O'BRIEN, P. J. (1980). Evidence for both local and central regulation of rat rod outer segment disc shedding. *Invest. Ophthal. Vis. Sci.* **19**, 1268–1273.

VAN NIE, R., IVANYI, D. & DEMANT, P. (1978). A new H-2 linked mutation, *rds* causing retinal degeneration in the mouse. *Tissue Antigens* **12**, 106–108.

YOUNG, R. W. & BOK, D. (1969). Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J. Cell Biol.* **42**, 392–403.

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