A new form of hereditary retinal degeneration in Wag/Rij rats. YIN-LOK LAI, ROBERT O. JACOBY, ALBERT M. JONES, and DAVID S. PAPERMASTER.

A spontaneous, hereditary, bilateral retinal degeneration affecting all adult animals in a closed, inbred colony of Wag/Rij rats has been discovered. The disorder is characterized by early onset and a slow progressive course. Early lesions are detected by one month in retinas which are otherwise fully developed. Destruction of the photoreceptor layer proceeds as more and more cells degenerate. Degeneration appears to begin in the photoreceptor cell body and only secondarily affects the outer segment. Furthermore, phagocytic activity of pigment epithelium remains intact until late in the disease. Endstage lesions include retinal degeneration produced by sodium iodate, Exp. Eye Res. 5: 86, 1966.


Retinal degeneration in mice and RCS rats has been especially well characterized. Sorsby and co-workers1 found that the distal segments of rods in C3H mice never fully develop. Thus retinal degeneration in these mice is primarily a failure in postnatal development of photoreceptors rather than a degeneration of fully developed photoreceptors. Retinas of RCS rats, on the other hand, appear to undergo normal postnatal development but degenerative lesions begin by two weeks of age and progress rapidly to complete degeneration at about two months.4 Lamellar membranes and excessive amounts of rhodopsin accumulate in the region between the tips of the outer segments and the adjacent pigment epithelium.5

Autoradiographic studies suggest the source of this material is undigested rod outer segment membranes and the basic defect appears to be a failure of pigment epithelial phagocytic function.1,2 Von Sallman and Grimes3 have reported progressive retinal degeneration in Osborne-Mendel rats, but ultrastructural and genetic studies have not been described.

We now report a new type of retinopathy which occurs in 100 per cent of animals in a closed inbred Wag/Rij rat colony. It is characterized by early onset, slow, progressive degeneration of photoreceptor cells, proliferation and vascularization of pigment epithelium, and migration of pigment epithelial cells into the retina. Endstage lesions closely resemble those of human retinitis pigmentosa.

Materials and methods. Breeding pairs of inbred Wag/Rij rats were initially obtained from the Radiobiological Institute T.N.O. (H.S.R.) Rijswijk, Netherlands, and a small colony was established at Yale by brother × sister matings. They are housed in a controlled environment room within a barrier facility and fed rat chow and hyperchlorinated water (9 p.p.m.) ad libitum. Cage racks are positioned at permanent locations in the room. Plastic breeder boxes without filter caps are positioned at specific levels on the racks and once a breeder pair is established, their location remains constant and they are exposed to the same light intensity throughout life. Weaning rats are handled in an identical manner. Ambient lighting is provided by ceiling-mounted fluorescent lamps regulated on a 12 hour on, 12 hour off cycle. Approximate light intensities are as follows: 65 foot candles (f.c.) 6 feet above the floor directly under a fluorescent light, 32 f.c. at cages positioned on the top shelf of each rack, 4 f.c. at the middle shelf, and 0.5 f.c. at cages located on the bottom shelf, 8 f.c. at the floor directly below a fluorescent light. A systematic analysis of the retinal dystrophy and light exposure is in progress. Several other rat strains maintained and bred under similar conditions had no evidence of retinopathy.

Fifty five male and female rats from 1 to 18 months of age were examined (see Table I). Animals were anesthetized with ether and perfused through the aorta with 2 or 4 per cent glutaraldehyde in either 0.1M cacodylate, pH 7.4 or 0.1M Sörensen buffer, pH 7.4.
Fig. 1. Development of degeneration in retinas of Wag/Rij rats. A, one-month-old rat: the retina is structurally normal, photoreceptor cell nuclei are arranged with about 12 cells per column. A photoreceptor cell (small arrow) is undergoing early degenerative changes including nuclear enlargement and dispersion of heterochromatin. Abbreviations: inner nuclear layer (In), outer nuclear layer (On), inner segment (is), outer segment (os), pigment epithelium (pe), early degenerating cell (small arrowhead), advanced degenerating cell (large arrowhead). x1,000. B, three-month-old rat: degenerating photoreceptor cells are more numerous and the outer nuclear layer has shortened to about 10 cells per column. Focal cellular swelling indicative of early degeneration (small arrowhead), and increased electron density indicative of advanced degeneration (large arrowhead) is seen in photoreceptor nuclei, inner segments, and neurons in the inner nuclear layer. Other segments and pigment epithelium are structurally normal. x1,000. C, six-month-old rat: there is a marked loss of photoreceptor cells with substantial reduction in the height of the outer nuclear column. Note photoreceptor cells (small arrowhead) showing typical early degenerative changes including nuclear enlargement, dispersion of heterochromatin, increase of cytoplasmic volume in both cell bodies and inner segments, and a general decrease of electron density. Spaces between rod outer segments are enlarged and filled with a finely granular material (seen clearly in Fig. 4, below). There is reduction in the total number of rods, the remaining outer segments are structurally normal. D, twelve-month-old rat: advanced degeneration with further reduction of cells in the outer nuclear layer (On) and inner nuclear layer (In). Inner segments (is) are shortened and disorganized and have marked variation in electron density. Outer segments (os) are markedly disorganized and fragmented. The pigment epithelium (pe) is structurally normal.

7.4, or 0.1M phosphate buffer, pH 7.4, at a constant pressure of 80 mm. Hg. Eyes were enucleated and anterior halves were removed by a transverse cut behind the limbus. Posterior halves were placed in glutaraldehyde fixative for two to three hours, washed briefly with buffer, and postfixed in 1 per cent buffered osmic acid at room temperature for two hours. Tissues were dehydrated in graded ethanol, cleared in propylene oxide, and embedded in Spurr's medium. Thin sections were stained with uranyl acetate and lead citrate, and were examined with a Zeiss EM 9 or a Philips 300 electron microscope.

Results. The development of the disease. The retinas of Wag/Rij rats developed to the adult form by one month of age. A few photoreceptor cells (less than 1 per cent) from one-month-old rats had early changes of degeneration (Fig. 1, A). By three months, bilateral, focal, degenerative changes appeared among greater numbers of photoreceptor cells and bipolar cells (Fig. 1, B). Changes became more widespread by six months and the thickness of the photoreceptor cell mu-
clear layer was reduced from 10 to 12 cells to about eight cells per column (Fig. 1, C). Photoreceptor degeneration progressed during the next six months and advanced degeneration was seen in most rats by 12 months (Fig. 1, D), and in all rats by 15 months, although only eight rats older than 12 months are included in this study, more than 50 aged Wag/Rij rats have been examined histologically and all have had comparable retinal degeneration. No sex differences were noted.

Degeneration proceeded as a marked, widespread decrease in the number of photoreceptor cells with disorganization of inner and outer segments. Endstage degeneration was characterized by retinal disorganization. Photoreceptor degeneration was accompanied by changes in the pigment epithelium. Pigment epithelial cells proliferated and migrated into the retina. Retinal vessels invaded the pigment epithelium and vitreous and migration of pigment epithelial cells into the retina was prominent. Pigment epithelial cells were detected between neurons of the inner nuclear layer and cuffed blood vessels penetrating into the retina (Fig. 2, A and B).

The development of lesions in photoreceptor cell and pigment epithelium. The progress of cytologic changes was similar in all photoreceptor cells regardless of the time of onset. Therefore, the following description will profile the typical pattern of cell degeneration.

Alterations in the photoreceptor cell body preceded changes in the inner and outer segments. The nucleus assumed an oval outline, heterochromatin became dispersed and marginated, and there was a marked increase in cytoplasmic volume (Figs. 1 and 3, A). Nuclear changes were accompanied by juxtanuclear electron-dense inclusions (Fig. 3, A). Advanced lesions were characterized by shrinkage of the cell body with a generalized increase in nuclear and cytoplasmic electron density (Figs. 1, B and 3, B).

Changes in the inner segment were detected prior to those of the outer segment. Early changes were characterized by an increase in cytoplasmic volume and a decrease in electron density of the
Fig. 2. Endstage lesions of retinal degeneration of Wag/Rij rat. A, fifteen-month-old rat: retina is scarred focally and there is proliferation of pigment epithelium (pe) and migration of pigment epithelial cells to the vitreous surface of the retina (arrow). Note vascularization (bv) of pigment epithelium and vascular invasion (bv) of vitreous (Vit). x2,000. Macrophages (m) are present in the vitreous. B, fifteen-month-old rat: despite complete loss of photoreceptor cells the pigment epithelium (pe) is structurally normal. No accumulation of excessive lamellar membrane has occurred. x2,000.
Fig. 3. Degeneration of photoreceptor cells in Wag/Rij rat. A, six-month-old rat: early changes in rod cell (small arrowhead) including dispersed chromatin, increased volume of cytoplasm in cell body, juxtanuclear electron-dense inclusions (i). Adjacent cells with more advanced changes have higher electron density and reduced cell volume (large arrowhead) or are structurally normal (arrow). ×8,200. B, six-month-old rat: a single cell is undergoing advanced degeneration (large arrowhead), characterized by a marked increase of nuclear and cytoplasmic electron density and reduction of cell volume. Outer segment (os) is structurally normal. Adjacent cells are similarly shrunk (large arrowhead), swollen (small arrowhead), or structurally normal (arrow). ×4,400.

inner segments (Fig. 1, C). Later, the affected inner segment shrunk and the electron density of the cellular matrix increased. The outer segment remained relatively normal, except that interdiscl spaces decreased (Fig. 3, B). Extensive degeneration of the outer segment was observed only after the photoreceptor cell body had largely been destroyed (Fig. 1, D). The pigment epithelium remained morphologically normal during early stages of photoreceptor degeneration (Fig. 1) and normal phagocytic activity was detected at all stages of degeneration (Figs. 1 and 4). The terminal stages of retinal degeneration were, however, accompanied by proliferation of pigment epithelial cells and their subsequent migration into the retina (Fig. 2, A and B).

Degeneration of neurons in the inner nuclear layer (Fig. 1, B) and ganglion cells (Fig. 1, D) was also observed. The progress of these lesions relative to the photoreceptor changes is currently under investigation.

Discussion. These studies indicate that inbred Wag/Rij rats develop a hereditary retinal degeneration characterized by an early onset and a slow progressive course. Early changes in photoreceptor cells are detectable by one month in retinas which are otherwise fully developed. Destruction of the photoreceptor layer proceeds as more and more cells undergo degeneration.

Previous studies of hereditary retinal degeneration in rats, particularly of the RCS strain, indicated that photoreceptor degeneration may begin in the outer segment and pigment epithelium. These workers showed that during breakdown of the outer segment, extracellular debris accumulated between the outer segment and pigment epithelium, apparently because of a defect in the phagocytic activity of pigment epithelium. In contrast, retinal degeneration in Wag/Rij rats appears to begin in the photoreceptor cell body and only secondarily affects outer segments. Furthermore, accumulations of lamellar debris are not seen at the outer segment-pigment epithelial interface except at ad-
Fig. 4. Phagocytosis of outer segment lamellae continues normally at the pigment epithelium-outter segment interface from a six-month-old Wag/Rij rat. A fragment of outer segment (ros) phagocytized by a pigment epithelial cell (pe) is seen in the center of the picture. Phagosome (ph) containing lamellar material and lysosomes (l) are present in the cytoplasm of the pigment epithelial cell. x10,000.

advanced stages when outer segments and photoreceptor cells are almost completely destroyed. Phagocytic activity of the pigment epithelial layer remains intact until late in disease. Degeneration in Wag/Rij rats is also a more chronic progressive lesion that mimics the temporal development of retinitis pigmentosa in man, whereas degeneration in RCS rats occurs very rapidly in young animals. Collectively, these findings suggest that photoreceptor cell degeneration in Wag/Rij rats proceeds by a different mechanism than in RCS rats.

The temporal and structural characteristics of the retinal degeneration in Wag/Rij rats indicate that it may serve as a useful model for study of retinitis pigmentosa in man.

From the Section of Comparative Medicine and Department of Pathology, Yale University School of Medicine, New Haven, Conn. This study was supported in part by United States Public Health Service Grants RR 00393-07, RR 05358-13, and EY 00845 from the National Institutes of Health and a Senior Research Fellowship from the National Retinitis Pigmentosa Foundation to Dr. Lai. Submitted for publication Aug. 13, 1974. Reprint requests: Dr. Yin-Lok Lai, Section of Comparative Medicine, Yale University School of Medicine, New Haven, Conn. 06510.

Key words: albino rat, hereditary degeneration, photoreceptor cell, pigment epithelium, retina, retinal degeneration, retinitis pigmentosa.

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