Retinal dystrophy in the rat—A pigment epithelial disease

Warren L. Herron, Byron W. Riegel, Orlo E. Myers, and Melvin L. Rubin

Rod outer segment renewal rate was studied by autoradiographic technique in both the Wistar rat (control) and the Royal College of Surgeons (RCS) dystrophic rat. The dystrophic rat showed normal renewal rate until age 18 days, whereupon further outer segment disc movement slowed and a previously established retrograde degeneration of outer segments progressed. The dystrophic rat differed from the normal in that it showed no evidence of phagocytosis of rod outer segment material by its pigment epithelium. Argument that the primary retinal defect is at the level of the pigment epithelium is presented.

Key words: retinal dystrophy, heredity, pathogenesis, retinal pigment epithelium, phagocytosis, retinal rod outer segment, retinitis pigmentosa, methionine, tritium, histopathology, rats, Royal College of Surgeons, retinal degeneration.

Inherited retinal degenerations are found in both man and animals. In man the pathogenesis of these diseases remain obscure. Early stages of the human degenerations have not been available for evaluation. Animals showing a late histologic picture similar to those found in human end-stage degenerations (e.g., retinitis pigmentosa) have been studied as experimental models of the human diseases.

Hereditary retinal degeneration in the RCS rat has been extensively studied since it was first reported in 1938. Dowling and Sidman extended our knowledge significantly. In studying the RCS rat they found that the electroretinogram is normal in development until age 18 days. Thereafter, a gradual deterioration in the ERG begins, and complete loss is observed by two months of age. Concurrent histologic study showed that almost from the onset of rod outer segment production, some disorganized material distal to the outer segments is present in the RCS rat (see Fig. 1). This material gradually builds up, even as the outer segments increase in size. At the same time, the rhodopsin content in the retina of the RCS rat increases significantly, becoming almost twice the normal amount in the 20-day-old animal. Retinal rhodopsin reaches its peak level at about 30 days, and thereafter falls off.

In 1963, Droz showed by autoradiography that protein incorporation in rods exhibits two phases. He described a transient,
rapidly diffusing protein which he called “exportable.” Additionally, he observed a slow-moving “sedentary” protein which persisted in the outer segment for days.

Recent work by Young3-5 has shown that the “sedentary” protein is actually incorporated into the rod outer segments, which are continually renewed. The rod outer segment “discs” are produced and move in a scleral direction until phagocytized by the pigment epithelium,6 the entire cycle taking approximately nine days in the 4- to 8-week-old Long Evans rat, which has a pigmented eye. The “movement” of the disc is caused by the continual production of new lamellated discs at the base of the outer segment.

Lamellated structures are also found within pigment epithelium of the albino rat. These are similar in appearance to the lamellated structures in the frog pigment epithelium which have been shown by Young and Bok6 to be phagocytized outer segments. These phagosomes have not been described in electron microscopic observations of the pigment epithelium of the dystrophic rat.7-8

The purpose of this study was to determine if there is an overabundant production of rod outer segment material in the RCS rat or if the pigment epithelium is inadequately removing distal outer segment material and, thus, is unable to balance the rate of production.

Methods

Animals. The dystrophic RCS rats were descendants of a breeding pair kindly given to one of us (W. L. H.) by Richard Sidman. Our rats are brown hooded and white bodied with albino eyes. Control animals also had albino eyes, since their rate of rod outer segment material production (shown to be directly related to light exposure)5 was more likely to be comparable. Wistar rats were used.

The laboratory temperature and periods of light
exposure of 10 hours per day were identical for both groups.

Schedule for histological sampling. Two series of animals were studied. Group A animals were injected with tritium-labeled amino acid at 10 days of age, since they first show outer segment material at this time. Group B animals were injected at 15 days of age. This day was chosen to find out what happens during the time when the RCS first shows abnormal retinal function. The times monitored after injection in each group are listed in Tables I and II for Groups A and B, respectively.

As an indicator of protein incorporation into the rod outer segments, we utilized a tritium-labeled amino acid, methionine. Generally tritiated D-L methionine (specific activity 162 to 320 mc. per mmole) was injected intraperitoneally in a dosage of 30 μc per gram. After enucleation, the freeze substitution technique for fixation (as described by Young5) was carried out using isopentane cooled with liquid nitrogen. After freezing, the eyes were placed in a 5 per cent solution of picric acid and ethanol for at least one week at −70° C. Two alcohol changes were carried out prior to placing the tissue in a refrigerator overnight to bring it to 4° C. The final absolute alcohol change was at 4° C. The tissue was then allowed to come to room temperature and embedded in paraffin.

The 5 μ sections were placed on precleaned slides coated with chrome alum and dipped into Kodak NTB-2 Nuclear Track Emulsion. Slides were sealed in light-tight boxes containing desiccant. The preparations were exposed from 4 to 18 weeks at 4° C. Development was carried out using Kodak Dektol for two minutes at 17° C. After development the sections were stained with Harris’ hematoxylin.

Results

In both the control and dystrophic animals, injected at 10 and 15 days of age, the diffuse transient "exportable" protein labeling appeared to proceed normally. Also, the incorporation of amino acid into "sedentary" protein synthesis for the renewal of rod outer segments was the same in both control and dystrophic animals. However, once incorporated, the subsequent movement of the outer segment protein discs differed markedly in these two species.

Group A—animals injected at 10 days of age (Fig. 2). Monitoring of the rate of rod outer segment production in control

<table>
<thead>
<tr>
<th>Time after injection of histologic samplings</th>
<th>Wistar (control)</th>
<th>RCS (dystrophic)</th>
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<td>½ hr.</td>
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<td>1 day</td>
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<td>14 days</td>
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<td>18 days</td>
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Wistar rats injected with methionine-H^3 at 10 days of age showed a rapid turnover of outer segment material, the entire process taking about 4 days. At 24 hours after injection, the label is primarily located at the junction of inner and outer segment material. At two days post injection, the label is seen in the distal outer segment
Fig. 2. Diagramatic representation of the position of the band of radioactivity in the layer of rods of normal (Wistar) and dystrophic rats (RCS) injected with methionine-H\(^3\) at 10 days of age. The pigment epithelium, location of the reaction band, and the thickness of the layer of rods are drawn to scale. (A) The thickness of the rod layers in the Wistar control rat is much less than in the dystrophic rat of comparable age. The radioactive label moves rapidly through the layer as the pigment epithelium phagocytizes the distal rod outer segment material. (B) The progressive thickening of the layer of rods in the dystrophic rat is graphically seen. The unchanging position of the radioactive label shows the failure of the pigment epithelium to remove old outer segment material.

material. Three days after injection, the label is less intense and is located in the most distal outer segment area. This suggests that much of the radioactive outer segment material has already passed through the pigment epithelium. Five days post injection, the area of intense radioactivity has already passed through the outer segment material. Later samplings show only a random distribution of radioactivity within the rod layer. Thus, we can interpolate that the normal complete outer segment disc renewal in a 10-day-old albino rat is between 3 and 5 days.

The 10-day-old dystrophic rat showed incorporation of the label into a reaction band within the proximal outer segment material in the 24 hour postinjection specimen. In subsequent time intervals studied, the label was seen to remain at about the same distance from the pigment epithelium (Figs. 3 and 4). There was no indication of phagocytosis of any outer segment material by the pigment epithelium. However,
Fig. 3. Here and in Fig. 4 are shown autoradiographs of dystrophic rat photoreceptor cells and pigment epithelium. The rats were 10 days old at the time of injection with methionine-\(\text{H}^3\). The pigment epithelium is at the top of both photographs; the photoreceptor nuclei are at the bottom. This photograph was taken 5 days after injection. The band of radioactivity is located in the disorganized swirls of extracellular material adjacent to the pigment epithelium. This band is quite distinct and has shown no movement from the time of its first production.

The total thickness of the retina has continued to increase. This increase in thickness is primarily due to the production of newer rod outer segment material which is not removed.

Fig. 4. Autoradiograph taken 18 days after injection (see legend of Fig. 3). A reaction of labeled protein can be seen adjacent to the pigment epithelium. The position of the label is unchanged from the 5 day postinjection specimen. However, the over-all thickness of the retina is 50 per cent greater than in the rat in Fig. 3. This is due to the continued production of new outer segment discs.
Fig. 5. Diagrammatic summation of the comparison of movement of the band of incorporated labeled amino acid in Wistar control (top) and RCS dystrophic (bottom) rats injected at 15 days of age. The diagrammatic symbols are identical to those shown in Fig. 1. T = time in days post injection of methionine-H\(^3\). A = age in days of rats from birth. The percentage of each layer occupied in each diagrammatic representation is based on measurements from the autoradiographs. In the RCS rat there is a varying width to the layer of rods. This diagram, drawn to proportion, indirectly shows this variance through the varying width of the pigment epithelial cells, which in reality remain relatively constant in size.

Fig. 6. Figs. 6 to 9 are autoradiographs of dystrophic rat photoreceptor cells and pigment epithelium. The rats were 15 days old at the time of injection. In all 4 figures, the pigment epithelium is at the top of the photographs; the photoreceptor nuclei are at the bottom. This photograph was taken 30 minutes after the injection. The radioactivity is concentrated in the inner segments of the rods and in the pigment epithelium. There is very little radioactivity in the area of the outer segments.
there was gradual thickening of the layer of rods caused by the continued production of new outer segment discs at the junction of inner and outer segments. This continued outer segment production caused the inner segments to be displaced from the radioactive label and the pigment epithelium.

**Group B — animals injected at 15 days of age (Fig. 5).** In the control group of 15-day-old Wistar rats, the methionine-H\(^3\) labeling in rod outer segments showed migration sclerally, the complete process taking just under 7 days, from injection until the radioactive incorporation band moved to the level of the pigment epithelium. This reaction band was in the proximal outer segment at 24 hours, and slightly more than a third of the way through the outer segments by three days. At 5 days it had traversed almost two thirds of the outer segment. In the 7 day specimen, most of the label had passed through the outer segments; the remaining label was located in the pigment epithelium and the most scleral portion of the rod outer segments. At 9 days, the band was no longer visible.

In the 15-day-old dystrophic rat, the incorporation of the labeled amino acid into the outer segment was normal. At 24 hours the band was at the base of the outer segments (Figs. 5 and 6). A distinct “reaction band” had traversed one fourth the distance of the organized outer segments by 72 hours (Fig. 7). Up until this point, the advancement of the band in the dystrophic rat was essentially the same as for the control animals. However, after 18 days of age (72 hours post injection), the band “movement” of labeled amino acid slowed significantly in the RCS rat. There was gradual progressive degeneration of the outer segments, which proceeded from the most distal outer segment material (located at the junction of outer segment and disorganized extracellular lamellae) in a retrograde fashion toward the inner segments, giving the retina extra thickness. There was evidence of some continued production of outer segment material. This material stains lighter on hematoxylin and

![Fig. 7. Autoradiograph taken 3 days after injection (see legend of Fig. 6). A reaction band can be seen in the proximal area of the rod outer segments. There is high concentration of background radioactivity in the choroid, outer nuclear layer, and pigment epithelium. Continued uptake of remaining available amino acid is seen in the inner segments.](image-url)
Fig. 8. Autoradiograph taken 14 days after injection (see legend of Fig. 6). A reaction band is seen at the junction of disorganized extracellular lamellae and the intact rod segments. This reaction band is still present at twice the time needed for complete photoreceptor outer segment renewal in a comparably aged control rat.

Fig. 9. Autoradiograph taken 43 days after injection (see legend of Fig. 6). A reaction band can be seen to persist amid the shrunken disorganized material which now occupies the space between the pigment epithelium and the remains of the outer nuclear layer. The shrunken outer plexiform layer can be seen, as well as bipolar cells. The pigment epithelial cells appear somewhat flattened. The background radioactivity at this time is negligible in the adjacent tissues.
eosin section and appears to be less dense than the older, more distal lamellae. At 11 days post injection (26-day-old RCS rat) the retrograde degeneration of outer segments had reached the area containing the labeled amino acid. Thus, the label was then located at the junction of the outer segments with the disorganized area. The label is located within the disorganized material at 14 days after injection (29-day-old RCS) (see Fig. 8). The tritium-containing material was still seen about midway between the external limiting membrane and the pigment epithelium at 43 days after injection of the label (Figs. 5 and 9). The rat, then 58 days old, showed no normal rod structure between the pigment epithelium and the external limiting membrane.

Discussion

The RCS rat shows a normal rate of outer segment movement until age 18 days. Thereafter, the movement of lamellae slows. The pigment epithelium shows no ability to "phagocytize" the rod outer segments, and labeled outer segment material is still present in a band in the last samplings taken in both age groups (18 days later in the 10-day-old series; 43 days later in the 15-day-old series). This is six times as long as the entire turnover period for a normal Wistar rat of the comparable age of injection (about 3 days in the 10 day series, and 7 days in the 15 day series).

Three factors indicate that the pigment epithelium is primarily responsible for the retinal degeneration in the dystrophic rat:

1. There is no indication that the outer segment material produced by the dystrophic rat is abnormal. Dowling and Sidman found that the rhodopsin in the dystrophic rat had an entirely normal spectrum. On electron microscopic evaluation of the 18-day-old dystrophic rat retina, these authors found the inner segments, ciliary apparatus, and rod outer segments to appear anatomically normal.

2. The development of the visual cells and the rate of outer segment production in both the Wistar and RCS rats is similar until 18 days of age, after which the RCS rat shows a cessation of significant movement of new lamellae. Therefore, there is no overproduction of rod outer segment material in the dystrophic rat.

3. The labeled amino acid is seen to still persist in the form of a "reaction band" amid the degenerated extracellular lamellae. Microscopic examination of the 10 and 15 day sequences failed to show any evidence that the pigment epithelium was removing any of the extracellular disorganized lamellae, either by decrease in the amount of extracellular lamellae or by progression of the labeled band to a position closer to the pigment epithelium. It appears that the RCS rat shows an absolute inability of its pigment epithelium cells to phagocytize rod outer segment material.

We have demonstrated that retinal dystrophy in the RCS rat is likely a disease of the pigment epithelium—more specifically, an inability to phagocytize. Perhaps an enzyme deficiency is the cause of the specific inability of the pigment epithelium to phagocytize. This area is deserving of further research.

It seems probable that the death of the visual cells in the RCS is the result of poor nutritional supply to these cells. We feel that this is caused by the build-up of extracellular lamellae, which serve to inhibit metabolic flow and remove the visual cell from its nutritional source, the choriocapillaris (Fig. 1).

Finally, a speculation is proposed. The similarity of the histologic picture in the end stage of human retinitis pigmentosa compared with the end stage RCS rat indicates the possibility of similar pathogenesis. If some human disease is also due to decreased ability of the pigment epithelium to phagocytize rod outer segment material, it might be possible to slow the rate of early clinical deterioration by slowing production of extracellular lamellae. For example, one could slow the destruction of intact rod outer segments by restricting light and, in addition, one might slow the
production of rod outer segments by inducing relative vitamin A deficiency. Vitamin A supplementation for retinitis pigmentosa, which has been so extensively tried in the past, may well have been the exact opposite of the steps needed to attempt to control the disease.

We would like to express our appreciation to Miss Carole Ames, Veterans Administration Hospital, Gainesville, Fla., for making this work possible by her excellent histologic assistance and selfless dedication to all aspects of this project.

REFERENCES