Outer segment production and removal in the degenerating retina of the dystrophic rat

Warren L. Herron, Jr., Byron W. Riegel, and Melvin L. Rubin

The production of rod outer segment material and its fate in the retina of the dystrophic rat was studied by autoradiographic technique. The pigment epithelium of the dystrophic rat shows an inability to remove its rod outer segment material. By 36 days of age, photoreceptor outer segment production in the dystrophic rat gradually ceases, although uptake of amino acids by the inner segment still appears normal. The first outer segment material which the developing dystrophic rat produces is still adjacent to the pigment epithelium 120 days later. Subsequently produced outer segment material is removed first apparently, suggesting that the removal of this material may be through the inner retina.

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

The dystrophic rat of the Royal College of Surgeons (RCS) strain has an autosomal, recessively inherited retinal degeneration. This rat has been extensively studied because it has been considered to be a possible experimental model of human retinal pigmentary degeneration. The RCS rat and human beings with retinitis pigmentosa share similar electroretinographic findings and both show a strikingly similar end-stage histologic picture. No human retinas with early retinitis pigmentosa have been histologically studied. The similarity of the retinal dystrophy in man and rat remains speculative.

Dowling and Sidman compared the developing RCS rat with the albino Harvard rat. They found that the retina of the dystrophic rat became markedly thickened prior to the degeneration of the photoreceptor cells. This increase in retinal thickness is caused by the build-up of a layer of disorganized extracellular material which is located between the rod outer segments and the pigment epithelium of the retina (Fig. 1). We have demonstrated in a previous report that this layer of disorganized extracellular material is rod outer segments which accumulate because of the inability of the pigment epithelium of the dystrophic rat to phagocytize its rod outer segment material.
The inability of the pigment epithelium of the RCS rat to phagocytize its rod outer segments is distinct from normal rats. Normal rats (and all other animals with rod photoreceptors so far reported) continually renew their rod photoreceptor outer segments by producing new outer segment "discs" at the base of the outer segments. The continued production of the new discs displaces the previously formed ones in a scleral direction, until the discs are eventually phagocytized by the pigment epithelium of the retina.

Having established that the pigment epithelium of the dystrophic rat does not phagocytize its rod outer segment material, we initiated two supplemental studies: (1) an evaluation of photoreceptor outer segment production during the phase of photoreceptor degeneration in the dystrophic rat; (2) an evaluation of the long-term fate of the outer segment material produced by the rods of the dystrophic rat.

Our findings are the subject of this communication.

Materials and methods

Animals. The dystrophic RCS rats were descendants of a breeding pair kindly given us by Dr. Richard Sidman.

Schedule for histologic samplings. See the Tables for samplings for Experiments 1 and 2.

Autoradiography. Our experimental method consisted of autoradiography with the injection of a labeled amino acid, generally tritiated methionine, to follow the course of its incorporation into visual cell outer segment protein. The dosage and methods are given in detail in a previous publication.

Experiments and results

1. Evaluation of photoreceptor outer segment production during photoreceptor degeneration in the dystrophic rat.

In the RCS rat, the electroretinogram shows abnormality at 18 days of age and subsequently degenerates to extinction. We had observed that the movement of outer segment material from the inner segment slowed at about this time. We set out to observe the course of photoreceptor disc renewal during the time of degeneration of the photoreceptor cells.

Rats ranging in age from 22 to 36 days were injected with labeled amino acid (see Table 1). They were killed at ten minutes and six days after injection.

Ten-minute post injection series. All the dystrophic rats injected between the age of 22 and 36 days showed accumulation of amino acid at the inner segments within ten minutes of administration of the tracer (see Fig. 2).
Fig. 2, A to C. For legend see opposite page.
Fig. 2. Autoradiographs of retinas of dystrophic rats. Note in all of these photomicrographs the pigment epithelium of the retina is above and the inner retinal layers are below. All show accumulation of the tritiated methionine in the pigment epithelium and the inner segments of the photoreceptor cells at 10 minutes after injection. (A) Injected at 22 days of age; (B) injected at 24 days of age; (C) injected at 27 days of age; (D) injected at 33 days of age; (E) injected at 36 days of age.

Six-day post injection series. Similar samplings were made six days after administration of the tracer into the rats injected between the ages of 22 and 36 days (Figs. 3 and 4). A distinct reaction band of labeled incorporated amino acid—an indication of continued production of new outer segment material—can be seen prominently in the rats injected as late as 27 days of age (see Fig. 3). A hint of a band can also be observed in the rats injected at 33 days of age (Fig. 4, A). Incorporation is visible near the inner segments in the animal injected with methionine at 36 days of age, but distinct banding is not seen (Fig. 4, B).

One must recall that each rat studied six days after injection had six days to...
Fig. 3. Autoradiographs of retinas of dystrophic rats. Note in all of these photomicrographs the pigment epithelium of the retina is above and the inner retinal layers are below. (A) Injected at 22 days of age; enucleated 6 days later; (B) injected at 24 days of age; enucleated 6 days later; (C) injected at 27 days of age; enucleated 6 days later. Outer segment reaction band is present in all of these.
produce newer outer segment material. As the age of the rat at the time of injection is increased, the labeled material six days later is seen to lie nearer and nearer to the inner segments. This is graphic proof of the slowing of production of outer segment material by the degenerating photoreceptors (see Figs. 3 and 4).

We concluded from this series that amino acid readily gets to the inner segments through 36 days of age. There is, however, evidence of progressively decreasing photoreceptor renewal becoming more apparent in the 33- and 36-day-old animals. This progressive decrease of photoreceptor renewal is evidenced by the increasing proximity of the label to the inner segments as the age of the rat increases and by the failure of the older animals to form a distinct reaction band of labeled protein.

II. Evaluation of the long-term fate of the outer segment material produced by the rods of the dystrophic rat. In normal rats, the photoreceptor cells start producing

Fig. 4. Autoradiographs of retinas of dystrophic rats. Note in both of these photomicrographs the pigment epithelium of the retina is above and the inner retinal layers are below. (A) Injected at 33 days of age; enucleated 6 days later. There is decreased accumulation of label in the proximal outer segment material. This picture is suggestive of a great decrease in outer segment renewal compared to earlier samplings. (B) Injected at 36 days of age; enucleated 6 days later. Incorporation is visible near the inner segment in the animal injected with methionine at 36 days of age. No distinct band is visible, indicating still further decrease in outer segment renewal.
Fig. 5. A to C. For legend see opposite page.
Fig. 5. Autoradiographs of retinas of dystrophic rats. Choroid and pigment epithelium are to the top of the photographs and the inner retinal layers are below. All animals were injected at 10 days of age. Note that the proximity of the label to the pigment epithelium does not change as the outer segment material gradually thins. Also, note that the outer nuclear layer progressively degenerates as time progresses. (A) Enucleated 40 days later; (B) enucleated 60 days later; (C) enucleated 80 days later; (D) enucleated 100 days later; (E) enucleated 120 days later.

rod outer segment material at about ten days of age. Normal albino rats injected with labeled amino acid at ten days of age take about four days for complete photoreceptor renewal—from production of outer segment material at the base of the outer segments to removal of this material by the pigment epithelium.

In the dystrophic rat, the photoreceptor cells also start producing outer segment material at about ten days of age. Thereafter this material accumulates, as it is not removed by the pigment epithelium. The distance between the inner segments and the pigment epithelium in the dystrophic rat becomes markedly greater than in the normal rat because of the accumulation of the degenerating outer segment mate-
Table II. Samplings for evaluation of the long-term fate of the outer segment material produced by the rods of the dystrophic rat

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<th>Age of rat at time of injection (days)</th>
<th>Time between injection and sampling (days)</th>
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The production of outer segment material ceases with the dystrophic rat at about 36 days of age. As the degeneration progresses, the photoreceptors and the outer segment material gradually disappear. In its end-stage histologic picture, the RCS rat shows the pigment epithelium adjacent to the inner nuclear layer. The layer of rods, the outer nuclear layer, and the outer plexiform layer have degenerated. This is similar to the end-stage histologic picture of retinitis pigmentosa in man.

We set out to observe the long-term fate of this persistent outer segment material produced by the dystrophic rat in the hope that we could learn something about how the retina reaches the end-stage histologic appearance.

As the animals grow older and the degeneration progresses, the area of disorganized protoreceptor material and the outer nuclear layer gradually become thinner (see Fig. 5). However, the proximity of the labeled outer segment material to the pigment epithelium does not change as time progresses. (Fig. 5). Eventually, at 120 days after injection, there are retinal areas where the inner nuclear layer is adjacent to the pigment epithelium. Other areas, where some disorganized outer segment material persists, show that this material is still labeled (Fig. 5, E). In these older rats where outer segment material persists, the area adjacent to the pigment epithelium still contains the radioactive label, while the outer segment material adjacent to the remnants of the outer nuclear layer is unlabeled (see Fig. 5, B to D).

These findings suggest to us that in the dystrophic rat, the pigment epithelium does not remove the outer segment material and that this material is removed by way of the inner retina until finally the bipolar cells are adjacent to the pigment epithelium.

Comment

The dystrophic rat continues to produce new rod outer segment material even as the photoreceptor cell degeneration is progressing. Uptake of labeled amino acid into the inner segment is seen through 36 days of age. There is no evidence that the build-up of extracellular material between the inner segments and the pigment epithelium markedly decreases the availability of amino acids to the inner segments. There is also no evidence that the inner segments lose their ability to accumulate these protein precursors.

Outer segment production is distinctly

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present through 27 days of age. Continued incorporation of amino acid into rod outer segment protein can be seen in the 33-day-old rats. The production appears much less distinct and less well organized in the 36-day old rats, and no definite band can be seen. This is evidence that outer segment production is terminating in the 36-day-old animals.

Labeled outer segment material produced at ten days of age is still present 120 days later in areas where outer segment material persists. The proximal outer segment material and outer nuclear layer are apparently removed prior to removal of the labeled protein adjacent to the pigment epithelium. This removal appears to be through the inner retinal layers. The pigment epithelium of the dystrophic rat never exhibits normal phagocytic removal of old outer segments.

REFERENCES