than in the inferior hemisphere of the eye (Table I). This asymmetry has also been observed in the one other normal pigmented strain (ACI) that has been examined for the feature. Because the rate of outer segment disc synthesis appears to be the same in outer segments of different lengths (Table II, Fig. 1), the generally accepted rod outer segment renewal time of 9 to 10 days for the rat11, 13 may vary. If the outer segments are longer than those in albino rats, as in the superior retinal hemisphere of pigmented rats, the total renewal time will be longer than 9 to 10 days, up to 14.6 days according to the present data. This situation resembles that of the rhesus monkey retina, in which the outer segment renewal rate is the same throughout the retina but the renewal time ranges from 9 to 13 days in different regions depending on the outer segment length.14

In pigmented control rats the temporal pattern and magnitude of disc shedding also appear to be the same in rod outer segments of different lengths (Table I, Fig. 2). This finding is consistent with the need for rod outer segment disc synthesis to be balanced by an equal amount of disc turnover in order to maintain any given outer segment length.

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From the Department of Anatomy, University of California, San Francisco, School of Medicine, San Francisco, Calif. 94143. This work was supported in part by U.S. Public Health Service Research Grant EY 01919 and by Contract EY-6-2147 and Core Grant EY 02162 from the National Eye Institute. Submitted for publication Nov. 18, 1980. Reprint requests: Dr. Matthew M. LaVail, Department of Anatomy, University of California, San Francisco, School of Medicine, San Francisco, Calif. 94143.

Key words: RCS rats, congenic strains, retinal dystrophy, rod outer segments, rods and cones, outer segment renewal

REFERENCES


RCS rat macrophages exhibit normal ROS phagocytosis. IGAL GERY AND PAUL J. O’BRIEN.

Peritoneal macrophages from RCS rats exhibit phagocytic capability equal to that found in macrophages from normal strains of rat. The similarity in phagocytic activity was found with rat or bovine rod outer segments as well as with dystrophic retinal debris. Thus the genetic defect in RCS pigment epithelium is not expressed in the macrophages, which are available to clear the debris layer that accumulates in dystrophic RCS retinas.

Inherited retinal degeneration in the Royal College of Surgeons (RCS) rat has been shown to be the result of a defect in the pigment epithelium (PE) cells.1-3 This defect may be related to the recognition of rod outer segment (ROS) membranes by PE cells because the phagocytosis of shed ROS tips is greatly impaired,4 although other materials such as carbon particles5 and polystyrene spheres4 are ingested readily by RCS PE cells.
LaVail \({}\) as well as Essner and Gorrin \({}\) have shown that macrophages (M\(\textphi\)) invade the layer of debris that accumulates in the RCS interphotoreceptor space as the degeneration progresses. However, Essner and Gorrin were unable to find evidence of phagocytosis of the debris by the M\(\textphi\) and raised the question as to whether the defect found in the PE cells was also expressed in the M\(\textphi\). Accordingly, we have compared the phagocytic activity of peritoneal controls (RCS-rf/i/ + or Sprague Dawley [SD]) and debris that accumulates in the RCS interphotoreceptor space as the degeneration progresses. Evidence of phagocytosis of the debris by the M\(\textphi\) was also expressed in the RCS-rf/i/ + kindred provided by Dr. Eileen Masterson (National Eye Institute).

Materials and methods

Rats. Male or female dystrophic, pink-eyed, tan-hooded RCS rats (RCS-PETH) were supplied by the National Institutes of Health breeding facilities and used at the ages of 3 weeks to 9 months. Control animals included normal SD rats from the NIH breeding facilities or unaffected control RCS rats (RCS-rdf+) kindly provided by Dr. Helen Hess (National Eye Institute).

M\(\textphi\) cultures. "Resident" M\(\textphi\) were obtained from untreated donor rats, and "induced" M\(\textphi\) were collected from rats injected intraperitoneally 3 or 4 days previously with proteose peptone broth (10%, NIH media unit, 50 ml/kg body weight). Rats were killed by CO\(\text{2}\), and their peritoneal cavities were washed with 100 to 200 ml Hank's balanced salt solution (HBSS, NIH media unit). After centrifugation (200 \(\times\) g, 7 min) the peritoneal cells were resuspended in M-199 medium (GIBCO, Grand Island, N.Y.) and recentrifuged two more times and were then resuspended in M-199 so that 2 ml contained the ROS from 2 or 3 retinas (13,962 or 21,720 dpm, as indicated). These ROS were used immediately or after a short storage at 4\(\circ\) C to insure that the plasma membranes would remain intact. Approximately half the ROS remained intact; the others were broken into large fragments.

Labeled RCS debris was prepared by intravitreal injection of RCS-PETH rats with tritiated leucine as described above. Eight animals were injected at 22 days of age; they were sacrificed 13 days later and the eyes were enucleated. Anterior segments were removed and the retinas were dissected. Both the retinas and the eye cups were rinsed with HBSS. Fragments of retina were allowed to sediment in a conical centrifuge tube. The supernatant debris suspension was removed and centrifuged for 10 min at 27,000 \(\times\) g. The ROS were resuspended and recentrifuged two more times and were then resuspended in M-199 so that 2 ml contained the ROS from 2 or 3 retinas (13,962 or 21,720 dpm, as indicated). These ROS were used immediately or after a short storage at 4\(\circ\) C to insinure that the plasma membranes would remain intact. Approximately half the ROS remained intact; the others were broken into large fragments.

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Labeled bovine ROS were prepared as previously described \({}\) by incubation of retinas with \((\text{H})\)choline (New England Nuclear, 80 Ci/m mole). Isolated ROS were stored at -20\(\circ\) C as pellets. Prior to each experiment pellets were suspended in M-199 so that 1 ml contained the ROS from about one twentieth of a retina (30,000 to 50,000 dpm, as indicated).

No fetal calf serum was present in any of the tested suspensions.

Uptake of \((\text{H})\)-ROS. Monolayers of M\(\textphi\) or PE cells were usually incubated in duplicate or triplicate with 1 or 2 ml aliquots (wells or dishes, respectively) of the labeled ROS at 37\(\circ\) C or 4\(\circ\) C. After incubation for varying intervals the monolayers were washed thoroughly with phosphate-buffered saline (pH 7.2) and were dissolved with 0.25 or 0.5 ml NaOH, 0.2N. The radioactivity retained in the cells was measured by scintillation counting of 0.1 ml aliquots added to 0.5 ml of NCS tissue solubilizer (Amersham, Arlington Heights, 1981).
Table I. Uptake of $^3$H-ROS by Mφ from dystrophic RCS and other strains of rats

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Rat strain</th>
<th>Type of Mφ*</th>
<th>ROS source</th>
<th>Incubation time (hr)</th>
<th>$^3$H-ROS uptake†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>SD</td>
<td>Resident</td>
<td>Bovine</td>
<td>1</td>
<td>15,376 (0.09)</td>
</tr>
<tr>
<td></td>
<td>RCS-PETH</td>
<td>Resident</td>
<td>Bovine</td>
<td>1</td>
<td>17,262 (0.08)</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>Resident</td>
<td>Bovine</td>
<td>3</td>
<td>32,210 (0.08)</td>
</tr>
<tr>
<td></td>
<td>RCS-PETH</td>
<td>Resident</td>
<td>Bovine</td>
<td>3</td>
<td>38,791 (0.06)</td>
</tr>
<tr>
<td>II</td>
<td>SD</td>
<td>Resident</td>
<td>Bovine</td>
<td>2</td>
<td>16,696 (0.08)</td>
</tr>
<tr>
<td></td>
<td>RCS-PETH</td>
<td>Resident</td>
<td>Bovine</td>
<td>2</td>
<td>13,768 (0.07)</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>Resident</td>
<td>Rat</td>
<td>2</td>
<td>18,088 (0.12)</td>
</tr>
<tr>
<td></td>
<td>RCS-PETH</td>
<td>Resident</td>
<td>Rat</td>
<td>2</td>
<td>15,260 (0.05)</td>
</tr>
<tr>
<td>III</td>
<td>RCS-rdy*</td>
<td>Induced</td>
<td>Rat</td>
<td>3</td>
<td>15,083 (0.15)</td>
</tr>
<tr>
<td></td>
<td>RCS-PETH</td>
<td>Induced</td>
<td>Rat</td>
<td>3</td>
<td>16,032 (0.20)</td>
</tr>
</tbody>
</table>

*Resident Mφ collected from untreated rats; induced Mφ collected from rats 3 days after injection with protease peptone.

†Data are presented as dpm/mg protein; parentheses denote protein content of the monolayer as mg/dish.

RCS = Resident Mφ from dystrophic RCS and other strains of rats. Like-wise, bovine ROS were ingested equally well by the two types of Mφ.

The phagocytic characteristics of rat Mφ and cultured chick PE cells were compared with bovine ROS being used as test particles (Table III). Both cell types exhibited time- and temperature-dependent uptake of bovine ROS that followed similar courses.

Discussion. These experiments demonstrate that Mφ from dystrophic RCS-PETH rats at all tested ages are as capable of phagocytizing ROS membranes or dystrophic retinal debris as are Mφ from control RCS-rdy*, SD, and BN strains of rats. Thus the genetic defect is not expressed in Mφ, and these cells are capable of clearing the ROS debris layer that accumulates in the dystrophic RCS retina. The failure of Essner and Gorrin to see ingested ROS debris in Mφ found within this layer is at odds with the observations of LaVail.
Table III. Uptake of 3H-ROS by MΦ and PE monolayers*

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>MΦ</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
<td>4°C</td>
</tr>
<tr>
<td>1</td>
<td>12,821 (0.14)</td>
<td>1186 (0.11)</td>
</tr>
<tr>
<td>2</td>
<td>20,846 (0.15)</td>
<td>883 (0.15)</td>
</tr>
<tr>
<td>3</td>
<td>32,307 (0.14)</td>
<td>1225 (0.14)</td>
</tr>
</tbody>
</table>

*Monolayers were made of resident MΦ from SD rats or chick PE cells. Bovine 3H-ROS were used in aliquots containing 90,220 dpm/dish. See Table I for more detail.

and could be a result of the use of pigmented (RCS-p+) rats in most of the experiments of Essner and Gorrin; the progression of disease is relatively slow in the pigmented eye. The results reported here, obtained with pink-eyed (RCS-PETH) rats, are consistent with LaVail’s observations.

Both resident and proteose peptone-induced MΦ were tested in the present study. Much of the available information concerning rat MΦ was obtained with induced MΦ, which are easy to collect and make a relatively pure population. In experiments not included here, proteose peptone-induced and resident MΦ of the same rat batch were compared, and in most cases the induced MΦ were found superior in their ROS phagocytic capacity. It is therefore noteworthy that both types of MΦ from the dystrophic RCS rats resembled their counterparts from the control rats in their ROS phagocytic capacity (Table I).

The similarities between MΦ and PE cells shown in Table III support the notion that PE cells carry many features of MΦ. Recent studies have shown that cultured PE cells, like MΦ, exhibit receptors for complement or the Fc component of immunoglobulin G. The possible role of these receptors in binding and ingestion of ROS by PE cells awaits further investigation. It is of interest that PE cells from Irish setters with rod-cone dysplasia showed three to four times fewer Fc receptors than did PE from control dogs. The relationship between this finding and the pathologic changes in the Irish setter is not known, however. Similar studies with RCS rat PE cells may provide useful information concerning the genetic defect of these cells.

We are grateful to Dr. Eileen Masterson for cultures of pigment epithelium and helpful discussions, Dr. Helen Hess for the RCS-rd8+ rats, Mr. James P. Alligood for preparing the rod outer segments, Ms. Julia Derr for technical assistance, and Ms. Joyce McIntyre and Mrs. Noreen Beavers for typing the manuscript.

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Key words: retinal dystrophy, RCS rats, rod outer segments, phagocytosis, macrophages, pigment epithelium

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11. LaVail MM and Battelle B-A: Influence of eye pig-

We have recently demonstrated that singlet oxygen-mediated photosensitization can cause an increase in blue fluorescence and covalent cross-linking in bovine lens crystallins in vitro. Because these changes closely parallel modifications known to occur in human crystallins during aging and cataractogenesis, it was suggested that singlet oxygen may play an important role in the processes in vivo. To confirm these results, obtained with photosensitizers free in solution, we now report results from experiments with a polymer-bound photosensitizer and with photophysically generated singlet oxygen. Singlet oxygen photodynamically generated by polymer-immobilized rose bengal produced the following modifications to human lens crystallins in vitro: covalent cross-linking, increased blue fluorescence, yellow pigmentation, and formation of heavy-molecular-weight aggregates. Singlet oxygen generated photophysically was shown to cause an increase in blue fluorescence and covalent cross-linking in human crystallins. These findings substantiate the hypothesis that singlet oxygen may play an important role in the changes seen in human lens proteins during aging and cataractogenesis.

In a recent report, we have described the effects of a photodynamic system using the photosensitizing dyes methylene blue, rose bengal, and riboflavin on isolated lens crystallins. Evidence was presented that implicated photodynamically generated singlet oxygen \( (^1O_2) \) in the production of nondisulfide covalent cross-links and blue fluorescence in bovine lens proteins in vitro. Because these effects are very similar to modifications known to occur in human lens proteins during aging and cataractogenesis, we suggested that photodynamically generated singlet oxygen may play an important role in lens aging in vivo.

A problem with the use of soluble dyes in such experiments is removal of the dye from the protein after irradiation. Furthermore, it is difficult to absolutely exclude the possibility of direct effects of the dye on the protein in such a system. To circumvent these problems, we now report photodynamic sensitization mediated by polymer-immobilized rose bengal, demonstrating with this system that in addition to production of covalent cross-links and blue fluorescent species, singlet oxygen can also mediate the aggregation and pigmentation of lens crystallins. In addition, utilization of a neodymium–yttrium aluminum garnet (Nd-YAG) laser to produce singlet oxygen photophysically has allowed us to unequivocally rule out the possibility that the changes we have observed are due to direct effects of a dye on the protein.

Methods. Human lens soluble protein was prepared by homogenization of decapsulated lenses in cold \( H_2O \) followed by centrifugation at \( 10,000 \times g \) for 30 min. The supernatant was dialyzed exhaustively against \( H_2O \), lyophilized, and stored at \(-20^\circ C \) until used.

A hydrophilic polymer-bound form of rose bengal (HP-rose bengal) was purchased from Hydron Laboratories Inc., New Brunswick, N.J., under the tradename Sensito II. This polymer is produced by copolymerization of chloromethylstyrene and the monothiacyrlyc ester of ethylene glycol, with the bis-methacrylate ester of ethylene as cross-linker. The polymer is subsequently heated with rose bengal to yield HP-rose bengal. Irradiations were carried out in dram vials stirred with small magnetic stirring bars to keep the HP-rose bengal in suspension. Fifteen milligrams of HP-rose bengal was used per milliliter of protein solution. Lens proteins were dissolved at a concentration of 2.5 mg/ml in phosphate-buffered saline (PBS), pH 7.5, and were irradiated with about 500 ft-c (1.7 mW/cm\(^2\)) of white light from daylight fluorescent lamps. There was no change in \( pH \) during the experiments, which were conducted at room temperature.

Gel chromatography was performed on a column of Bio-Gel A5M (1 by 90 cm). Samples were eluted by gravity, and absorbance at 280 nm was automatically monitored by a Uvicord II ultraviolet monitor (LKB).

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) was performed as previously described.

The experimental techniques and apparatus for the photophysical generation of \( ^1O_2 \) have been described previously. Fluorescence measurements were made on an Aminco SPF-500 corrected-spectra spectrofluorometer. Amino acid analyses were made on a Beckman Model 119C by standard procedures after hydrolysis with methane sulfonic acid.

Results. As shown in Fig. 1, HP-rose bengal-mediated photosensitization gave effects similar