Subretinal photoreceptor cells have been observed in normal rat retinas of all strains which we have examined, from young developing rats to those 3 years of age. The number and distribution of subretinal photoreceptor cells have varied among individual rats as well as from different regions in the same retina. They are more frequently observed in either very young developing retinas or aged retinas. Within the retina itself, the presence of subretinal photoreceptor cells is most commonly observed in the peripheral retina and in areas around the optic disc.

Two main stages in the movement of photoreceptor cells into subretinal space can be distinguished. In the first stage, the cell bodies of these photoreceptor cells are still located within the outer nuclear layer, but their nuclei appear to be sliding through the outer limiting membrane, giving them an hourglass shape. The inner and outer segments of the cells are still present but are apparently altered. In the second stage, these subretinal photoreceptors have lost all connections with the outer nuclear layer, and the cells are present entirely within the subretinal space. The cells have nuclear morphology identical to that of the cells in situ, but their synaptic portions and inner and outer segments are either highly modified or completely abolished. Cytological evidence indicates that these subretinal photoreceptor cells are not normal. The abnormalities of these cells might have occurred during or even before the outward movement process. The mechanisms and biological significance of this phenomenon are not yet certain; however, it is undoubtedly one of the important factors contributing to the gradual loss of photoreceptor cells in the normal rat retina.

**Key words:** rat, retina, subretinal space, photoreceptor movement, pathology, cell loss, cell motility, age

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A gradual reduction in the photoreceptor cell population has been observed in rat retinas raised under cyclic fluorescent light intensities of less than 1 ft-cd. These retinas were characterized by a progressive thinning of the photoreceptor cell layer without the presence of obvious nuclear pyknosis among the photoreceptor cells. The inner and outer...
Table I. Frequencies of subretinal photoreceptor cells in rat retinas

<table>
<thead>
<tr>
<th>Age</th>
<th>No. examined</th>
<th>Percentage of positive findings</th>
<th>Range of cells present per section</th>
<th>Average of subretinal photoreceptor per section</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 wk.</td>
<td>6</td>
<td>83</td>
<td>81</td>
<td>1.15</td>
</tr>
<tr>
<td>3 wk.</td>
<td>5</td>
<td>100</td>
<td>93</td>
<td>1.13</td>
</tr>
<tr>
<td>1 mo.</td>
<td>10</td>
<td>30</td>
<td>20</td>
<td>1.2</td>
</tr>
<tr>
<td>3 mo.</td>
<td>10</td>
<td>60</td>
<td>39</td>
<td>1.5</td>
</tr>
<tr>
<td>12 mo.</td>
<td>8</td>
<td>100</td>
<td>100</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Segments of the surviving photoreceptor cells were morphologically normal. The histopathology of these retinas was apparently different from retinal degeneration induced by exposure to either high-intensity short-duration light or low-intensity continuous light. In the former lesion, retinal damage was characterized by extensive photoreceptor pyknosis, whereas the latter lesion was characterized by initial early stratification and fragmentation of photoreceptor inner and outer segments. Although the reduction in photoreceptor population in those retinas was assumed to be age-related and light-associated, there was no evidence which demonstrated that low-intensity cyclic fluorescent light actually killed photoreceptor cells, nor was there provided any clue concerning the fate of the missing photoreceptor cells.

The purpose of this article is to present a new and important general phenomenon in the rat retina, i.e., outward movement of the photoreceptor cells into the subretinal space, and to discuss its possible significance.

Materials and methods

Our laboratory has carried out experimental studies on the rat retina. We have examined hundreds of retinas from various strains of normal rats, including Sprague-Dawley, Fischer, DA, and Long-Evans strains, and from rats of various ages from newborn to 3 years.

Rats were routinely perfused through the left ventricle and aorta with 3% glutaraldehyde in 0.1M phosphate buffer while under deep anesthesia from an intraperitoneal injection of sodium pentobarbital. Eyes were dissected immediately behind the limbus into two parts. The posterior eyecups were fixed in the 3% glutaraldehyde and phosphate buffer for 2 to 3 hr, then postfixed in 1% osmium tetroxide in phosphate buffer for 2 hr. After dehydration in graded ethanol, the tissues were cleared in toluene and embedded in Surr’s medium. Sections (1 μm) of the hemispheres of the eyecup, including a full length of the retina between two sides of the ora serrata and optic disc, were stained with azure II–methylene blue for light microscopy. Selected areas were thin-sectioned for electron microscopy.

Results

The presence of photoreceptor cells outside the outer nuclear layer and in the subretinal space is a general phenomenon in normal rat retinas. These subretinal photoreceptor cells were observed in all strains with a high frequency, approximately 50% of the normal rat retinas, and they were observed more frequently in very young developing retinas and in aged retinas. The number and distribution of the subretinal photoreceptor cells varied among individual rats and from different regions in the same retina. Serial 1 μm section of retinas revealed that these subretinal photoreceptor cells were not evenly distributed in all retinal regions. They were observed more frequently in the peripheral retina and around the posterior pole. Groups of subretinal photoreceptor cells observed in a given region of the retina would disappear in tissue section obtained several micrometers away from the previous one. Preliminary quantitation of the number of subretinal photoreceptor cells from randomly selected, normal rat retinas further confirmed that the presence of subretinal photoreceptor cells is truly a general phenomenon (Table I). Subretinal photoreceptor cells were observed in 30% to 100% of the normal rat retinas, de-
Subretinal photoreceptor cells were frequently found in the developing retinas of 2 to 3 weeks of age (80% to 100%), but in young adult rats of 1 to 3 months of age the presence of positive findings of subretinal photoreceptor cells was remarkably less (30% to 60%). By the age of 12 months, the presence of subretinal photoreceptor cells appeared to be most severe (100%). Furthermore, the frequency of subretinal photoreceptor cells found in retinal sections and the extent of their presence appear to be closely associated with the age of the animal.

Two main forms of subretinal photoreceptor cells could be observed in these retinas. In the first form, the cell bodies of the photoreceptors were still located in the outer nuclear layer but their nuclei appeared to be sliding through the outer limiting membrane and into their inner segments. In the second form, the photoreceptor cells were present in the subretinal space and had lost all connection with the outer nuclear layer. These two forms were apparently different stages of the same phenomenon.

The photoreceptor cells in the subretinal space had a nuclear morphology identical to
Fig. 2. Modified photoreceptor cell (D) is shown in the subretinal space between the normal photoreceptor outer segments (Os) and the retinal pigment epithelium (Ep). The typical synaptic spherule, inner segment, and outer segment are abolished. Three piles of modified and disoriented outer segment disks (Ao) are shown in the modified cytoplasm of the subretinal photoreceptor cell in the lower right corner of the micrograph. (X20,000.)

that of the photoreceptor cells in the outer nuclear layer, but the synaptic bodies, inner segments, and outer segments of the subretinal photoreceptor cells were either highly modified or completely abolished. The cytoplasmic volume of the subretinal photoreceptor cells was also remarkably decreased (Figs. 1 to 3). Modified inner segments, piles of disorganized outer segment disks, and connecting cilia were observed in the subretinal photoreceptor cells, indicating that these cells may have the specialized structures of other photoreceptors although they are highly modified (Figs. 1 and 2). No morphologically normal photoreceptor cells with intact synaptic body and inner and outer segments were found in the subretinal space of the rat retinas.

When the descending nuclei of the photoreceptor cells were observed at the level of the outer limiting membrane, the nuclei of such cells were often shaped like an hourglass, appearing as if they were sliding through the outer limiting membrane and into the inner segments. This occurred without abolition of the desmosomal structure at the outer limiting membrane. The inner segments of such photoreceptor cells were apparently altered. They had become shorter than their neighboring photoreceptor inner segments. Their outer segments had also become shorter, having fewer, smaller, and
Outward movement of photoreceptor cells

Fig. 3. Photoreceptor cell with descending nucleus (Ds) is shown in the left-hand side of the micrograph. This nucleus is shaped like an hourglass, appearing as if it were sliding through the outer limiting membrane and into the inner segment. There is no abolition of the desmosomal structure (arrow) at the outer limiting membrane (large arrow). The inner segment (Ai) and the outer segment (Ao) of this cell are shorter than normal and there are fewer, smaller, and disoriented outer segment disks. A highly modified subretinal photoreceptor cell (D) is shown in between the photoreceptor inner segments (Is). (×34,000.)

disoriented disks (Fig. 3). Necrotic photoreceptor cells were also observed in the subretinal space of rat retinas.

Discussion

Presence of photoreceptors in the subretinal space appears to be a general phenomenon of the normal rat retina which may result in a decrease in the photoreceptor cell population of the outer nuclear layer. It has been observed in all strains of rats and in about half the normal retinas we examined, with the peripheral retina and the area around the posterior pole showing the greatest number of subretinal photoreceptor cells.

Photoreceptor cells have been shown apparently descending through the outer limiting membrane, as well as entirely within the subretinal space, eventually descending to the apical surface of the pigment epithelium. While descending through the outer limiting membrane, the nuclei of such a displacing cell may often take on the appearance of an hourglass, with the still intact desmosomal complexes of the outer limiting membrane forming the area of constriction. The subretinal photoreceptor cell may often possess highly modified inner and outer segments as well as a nuclear structure identical to that of photoreceptors of the outer nuclear layer.
The presence of such specialized structures gives evidence concerning the origin of these subretinal cells.

The exact mode by which photoreceptor cells are displaced remains unclear. There is an argument suggesting that these cells are probably pushed outward from the outer nuclear layer. Only two factors can be considered in this aspect. The first is the intraocular pressure which acts on all retinal cells. This pressure cannot be selectively applied to an individual photoreceptor cell, and, furthermore, unless the involved photoreceptor disconnects its synapsis with the second retinal neurons, it would probably be difficult to push such a cell from its position. The other factor, a possible pressure exerted by surrounding Müller cells, also seems very unlikely. It has not been demonstrated that Müller cells are capable of contracting and squeezing the photoreceptor cells, and we did not see any evidence to support this idea.

Movement of photoreceptor inner segments during light/dark adaptations has been documented in fishes, amphibians, and birds. The length of the photoreceptor inner segments depends on a number of factors other than light, including temperature and retinal acidity.\textsuperscript{22-27} It is generally accepted that cytoplasmic contraction results from activities of the actomyosin of the cell\textsuperscript{28} and that elongation is produced by microtubules.\textsuperscript{29} Photoreceptor inner segments in the rat retina are claimed to contain high concentrations of myosin and actin and are thought to be able to perform certain motile activities.\textsuperscript{30} Despite the obvious evidence such as the presence of microtubules, actin, and myosin in the photoreceptor inner segments of the rat retina, there are no experimental data in the literature proclaiming movement of photoreceptor inner segments during light/dark adaptations in rat retinas. Whether or not the movement of photoreceptor cells into the subretinal space is an active or passive procedure is not yet clear.

Cell movements in other types of cells are usually preceded by projection of pseudopods and flow of the nucleus in the direction of movement. However, photoreceptor cells are among the most specialized cells in the body, and highly specialized areas such as synaptic bodies and outer segments are polarized at opposite ends of the cell. Unless these specialized structures were abolished or radically modified and the cells remained viable enough to move away to other locations, one would not expect to find pseudopods from the subretinal cells. On the other hand, the descending nucleus moving into the inner segment is well demonstrated in Fig. 3. This nuclear movement implies that the involved photoreceptor might have participated in the process of outward movement.

The question concerning whether or not the subretinal photoreceptor cells are normal during or even before the processes of cell displacement is of critical importance. Overwhelming evidence indicates that these cells are not normal. The shorter inner segment as well as the disorientation and the reduction of both size and number of outer segment disks in the photoreceptor cell with descending nucleus indicate that there is a diminution of cytoplasmic volume and a derangement of metabolic activities in the cell. These abnormalities demonstrate that the subretinal photoreceptor cells are not normal during the stages of subretinal placement or that they might be abnormal even before the beginning of the outward-displacing process. The higher degree of morphological modification observed in the subretinal photoreceptor cells indicates that these cells were in more advanced stages of subretinal movement than the cells with descending nuclei.

The burden of attempting to find a morphologically normal photoreceptor cell in the subretinal space is tremendous because the morphology of a cell reflects the physiological state of the cell, which is in turn influenced by the surrounding physical and biochemical environment. The subretinal photoreceptor cells have lost their synaptic connection with the second retinal neurons, have severed the biochemical exchanges with the Müller cells, and are placed in a biochemically and physically new environment. These functional and environmental changes are sufficient to cause morphological and biochemical modifications...
of the photoreceptor cells. Necrosis of photoreceptor cells in the subretinal space have also been observed.

Although the mechanisms and biological significance of outward movement of the photoreceptor cells into the subretinal space are not yet clear, preliminary quantitation of subretinal photoreceptor cells indicated that the frequency and severity of subretinal photoreceptor cells appear to be closely associated with the age of the animal; furthermore, the net result of this phenomenon is quite certain. It contributes in some extent to the gradual reduction in the population of photoreceptor cells in aged rat retinas.

Finally, there was a speculation arguing that various forms of white blood cells might be able to move freely through the subretinal space and thus might be confused with the subretinal photoreceptor cells. The general understanding and my personal experience indicate that both lymphocytes and granulocytes are not normal constituents in the subretinal space of the normal retina. However, they appear massively when inflammatory, degenerative, or immune responses are involved. I have observed three types of cells in the subretinal space of the normal rat retinas: the pigment epithelial cells (or pigment epithelial macrophages), the monocytes (or macrophages), and the subretinal photoreceptor cells. The monocytes (or macrophages) are smaller than the pigment epithelial macrophages, with irregular nuclear profiles and remarkable heterochromatin distributed marginally. These cells tend to be irregular in shape, with cell processes extending in different directions, and may or may not contain phagosomes. The morphology of monocytes (or macrophages) is so different from that of the photoreceptor cells that it is almost impossible to confuse them with subretinal photoreceptor cells. The characteristic feature of a small lymphocyte is a relatively large nucleus surrounded by a thin layer of cytoplasm. The nucleus is nearly spherical, but on one side it has a more or less obvious indentation. The chromatin forms a thick layer at the nuclear envelope and has several darkly staining masses in the interior. All lymphocytes have prominent nucleoli. The rod cells of the rat retinas have round nuclei with large centrally located chromatin mass(es) and possess specific cell structures which should not be confused with lymphocytes.

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REFERENCES
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**Erratum**

Abstract 19, page 213 of 1980 ARVO abstracts, should have three authors listed. The correct entry is: Reaction time, detectability, and the oblique effect—Thomas R. Corwin, Duane O. Bowker, and Marc B. Mandler.