A new form of retinal lesion has been observed in rats. It is characterized by (1) inward folding of the photoreceptor cell layer (outer nuclear layers); (2) microretinal detachment; (3) thinning of inner retinal layer; (4) loss of Müller cells in the lesion; (5) in most cases, normal retinal surface contour, and (6) no increase in total retinal thickness. Remarkable strain differences were noted. In Sprague-Dawley rats, the incidence increases with age, and lesions are more often located in the peripheral one third. On the other hand, in rat strains having known hereditary retinal disorders (Wag/Rij and Wag/Long-Evans), the incidence of lesions decreases with increasing age of observed specimens, and the lesions are more frequently located in the posterior one third. These new forms of retinal lesions differ from retinal folds found in the far periphery of the developing retina. Our findings suggest that loss of Müller cells in the lesions initiates this disorder. Invest Ophthalmol Vis Sci 26:771–774, 1985

Retinal lesions resembling retinal folds have been repeatedly attributed to be congenital in origin1–3; to be associated with irradiation in the human fetus,4 rabbits,5 and rats6; and to be associated with virus-induced retinal dystrophy in dogs.8 However, these reports actually dealt with different types of retinal lesions, from retinal dystrophies to true retinal folds. We report here a new form of spontaneous retinal lesion in rats which is an incomplete folding of the outer nuclear layer and has been characterized morphologically, temporally, and spatially. The significance and possible pathogenesis of these lesions are discussed.

Materials and Methods. Fifty-eight rats having retinal lesions were analyzed in this study. They were from various strains, including 28 Wag/Rij, 12 Wag/Long-Evans, 15 Sprague-Dawley, and 3 RCS. A breeding pair of inbred Wag/Rij rats were initially obtained from the Radiobiological Institute T.N.O. (HSR) (Rijswijk, Netherlands), and a small colony was established at Yale University. Other strains of rats were obtained from Charles River Breeding Laboratories (Boston, MA) at the age of 2 weeks. All rats were housed in a controlled environment room within a barrier facility in conformity to the ARVO Resolution on the Use of Animals in research. Ages of rats in each strain ranged from 2 weeks to 1.5 yr. Ambient lighting was provided by ceiling-mounted 40 watt fluorescent lamps regulated on a 12-hr-on, 12-hr-off cycle. The rats were never exposed to light intensities over 35 foot candles.

The animals were killed by an intraperitoneal injection of sodium pentobarbitol. Each animal was then perfused with 3% glutaraldehyde in 0.1 M phosphate buffer; perfusion pressure was maintained at about 60 mmHg. Immediately after perfusion, the eyes were removed and dissected into two parts just behind the limbus. The posterior parts of the eyes were fixed in the same buffered fixative for at least 3 hr. Tissues were then postfixed in 1% osmium tetroxide phosphate-buffered solution for 2 hr. The tissues were dehydrated in graded ethanol, cleared in toluene, and embedded in Spurr's medium.

For light microscopy, sections 1 μm thick, including a full length of the retina between two sides of the ora serrata and optic cup, were made. A minimum of eight serial sections were cut from each eye cup. The sections were stained with azure II-methylene blue. The retina in each section was divided into three zones: peripheral, middle, and posterior. The findings in each section were charted according to the location and the shape of the lesion. All retinal lesions were photographed. The size, shape, type, and thickness of retina were documented. Results were tabulated separately for each strain of rat.

Some areas containing retinal lesions were prepared for electron microscopic studies.

Results. Retinal lesions observed in rats can be categorized in two forms, according to their morphology. An early and less-severe form of lesion, characterized by the presence of a minute, elevated tent-shaped (Δ) space between the neuroretina and the underlyling retinal pigmented epithelium (RPE), is termed A-form lesion (Fig. 1). At the lesion, the outer nuclear layer with its photoreceptors is detached from the RPE and folds inward against the neighboring inner nuclear layer. The inner retinal layers in the lesion are often reduced in thickness. In general, the retinal contour is not altered, and no folding is observable in the retinal surface. Although the number of neurons in the inner retina is reduced, no marked change was found in the cytology of the surviving neurons in the inner retina. Most of the outer limiting membrane was found intact (Fig. 2).

In the subretinal space created by these folds, numerous macrophages were observed and found to contain a large amount of phagocytized outer segment material (Fig. 2).

More severe and extensive are “T”-form lesions. They are characterized by later expansions of the apical subretinal space at the outer and inner nuclear levels, thus creating a T-shaped or mushroom-shaped
in the outer nuclear layer and loss of outer limiting membrane in the lesion, were observed (Fig. 4).

The incidence, types, and locations of these retinal lesions in different strains of rats are shown in Table 1.

In 28 Wag/Rij rats (17 male, 11 female), 50 lesions were found. Of these, 32 were of A-form and 18 were of T-form. In 18 of these animals, the lesions were found in only one eye; 10 animals had lesions in both eyes. In 12 Wag/Long-Evans rats (6 male, 6 female), 22 lesions were found, of which 21 were of A-form and only one was of T-form. In four of these animals, the lesions were found in only one eye; eight animals had lesions in both eyes. In both of these strains, most lesions were distributed in the posterior third of the retina. The incidence of lesions in these strains, in general, decreased with increasing age of the animal.

In 15 Sprague-Dawley rats (9 male, 6 female), 29 retinal lesions were found, of which 26 were of A-form and three were of T-form. In 11 of these animals, the lesions were found in only one eye; four

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Fig. 1. “A” form lesions found in rat retinas. a, An early and less severe lesion which is characterized by the presence of a minute, elevated tent-shaped (Δ) space between the neuroretina and underlying retinal pigmented epithelium. In general, the retinal contour is not altered, b and c are more progressed “A” form lesions. The thickness of inner retinal layers is often reduced (×100).

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space in the lesion (Fig. 3). The inner and outer segments of the photoreceptor cells in the lesions are remarkably altered. They are shorter and have distorted disk membranes. Besides outer and inner segments, subretinal photoreceptors and macrophages were routinely found in the subretinal space of these folds. The cellular debris of degenerating photoreceptors appears to be either in the cytoplasm of the macrophages or in close proximity to these cells (Fig. 2).

The Muller cell nuclei in these folds and in an equal area on both adjacent sides of the folds were counted. The number of Muller cells per unit area in the retinal lesions decreased. Furthermore, occasional marked loss of Muller cells in the lesions, accompanied by dissociation of photoreceptor cells

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Fig. 2. Electron micrograph of subretinal space created by retinal lesions. Most of the outer limiting membrane (arrows) remains intact. The cytoplasm of a macrophage (m) contains cellular debris of degenerating photoreceptors. is: inner segments; os: outer segment (×8,000).
animals had lesions in both eyes. In this strain, most A-form lesions were located in the peripheral third of the retina. The incidence of lesions in these rats increased with increasing age of the animals. In three RCS rats (2 male, 1 female), 11 retinal lesions were found. In two of these animals, the lesions were in only one eye; one animal had lesions in both eyes. All of these lesions were of A form and most were located in the middle zone. Many retinas examined had more than one lesion. No sex-related differences was found in these studies.

**Discussion.** In this study a new form of retinal lesion was characterized temporally, spatially, and histopathologically at both light and electron microscopic levels. These retinal lesions were formed by inward foldings of the outer nuclear (photoreceptor) layer, with a micro (minute) retinal detachment, and accompanied by reduction in thickness of the inner retinal layers overlying the lesion. This abnormality was associated with a relative loss of Muller cells in the lesion.

The retinal lesions developed early as microretinal detachments with an A-shaped subretinal space and inward folding or invagination of the photoreceptor layer. Consequently, the inner retinal layers became thinner, the apical area of the subretinal space in folded lesions underwent lateral extension, hence, showing a T- or mushroom-shaped lesion in cross-section. In these lesions marked loss of photoreceptor cells was observed. Although outer and inner segments of photoreceptors were present in the subretinal spaces of these T form lesions, some of these segments showed degeneration, and some were phagocytized by the macrophages. To evaluate the full extent of these folds, serial sections were examined. In a histologic sense, these lesions are not a complete or usual form of retinal folds as have been described in the literature, but are rather an inward folding of the outer nuclear (photoreceptor) layer. Despite the presence of microretinal detachment and the folding of the outer nuclear layer, the absence of increase in distance between the RPE and inner limiting mem-

**Fig. 3.** a, b, More severe “T” form lesions found in rat retinas. These lesions are characterized by lateral expansions of the apical subretinal space creating a “T” or mushroom-shaped lesion. The thickness of inner retinal layers is often reduced in these lesions (×100).

**Fig. 4.** Photomicrograph of a retinal lesion. a, Showing disrupted outer nuclear layer (arrows) (×100). b, Higher magnification of outer retinal layers associated with retinal lesion. Spaces (arrows) are caused by the loss of Muller cells (×630).
Table 1. The incidence, types, and distribution of retinal lesions in different strains of rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of animals</th>
<th>Male</th>
<th>Female</th>
<th>No. of lesions</th>
<th>One eye</th>
<th>Both eyes</th>
<th>Distribution of type &quot;A&quot; lesions</th>
<th>Distribution of type &quot;T&quot; lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wag/Rij</td>
<td>28</td>
<td>17</td>
<td>11</td>
<td>50</td>
<td>18</td>
<td>10</td>
<td>Periph 10 Mid 8 Post 14</td>
<td>Periph 3 Mid 3 Post 1</td>
</tr>
<tr>
<td>Wag/LE</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>22</td>
<td>4</td>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SD</td>
<td>15</td>
<td>9</td>
<td>6</td>
<td>29</td>
<td>11</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RCS</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>—</td>
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</table>

* Many of the retinas examined had more than one lesion.

brane precludes subretinal fluid accumulation as a pathogenic cause of the lesions. Furthermore, the retinal surface contour was maintained in most of these lesions, and we did not observe any preretinal or extraretinal factors that could be attributed as the cause of them. On the other hand, Muller cells are well known for their role in biochemical interaction with the retinal neurons and as a skeleton of retina. Reduction of their number in these lesions may indicate a possibility of Muller cells' involvement in the pathogenesis of the lesions.

These new forms of retinal lesions differ from retinal folds found in the far periphery of the developing retina, in which the folds are caused by relatively rapid expansion of the surface dimension of the neuroretina in comparison with rate of development in the uveal coat. The neuroretinal growth in this case exceeds the expansional rate of outer (uveal and scleral) coats of the eye ball. Further, these transitional retinal folds were found mainly in young rats and had never been shown to acquire a T-shaped fold or have a micoretinal detachment.

As the fixation artifacts usually cause an extensive detachment of retinas from the sclera and RPE, the possibility of this was minimized by perfusion of animals while they were alive. Any damaging effect of light on retinas was also minimized. The rats in this study were never exposed to light intensities over 35 foot candles or to continuous room illumination over 12 hr at a time.

There are very obvious strain differences. In normal Sprague-Dawley rats, these lesions were observed more often in the peripheral zone than the posterior zone, and their number increased with increasing age. In rat strains having well known hereditary retinal degeneration (such as Wag/Rij and RCS), the lesions were more frequently located in the posterior zone of the retina, but their incidence decreased with increasing age. In RCS rats, few retinal lesions were observed, because the photoreceptor layer in these rats disappears by 1 month of age. It is interesting to note that T-form lesions in the posterior region were frequent in only Wag/Rij rats.

Key words: rats, retinal folds, subretinal space, photoreceptor cells, Muller cells, macrophages

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