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Lens Reconstruction After Mature Cataract in SCR Rat

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Purpose: To conduct a long-term observation study of SCR rats that had developed a mature cataract at 11 weeks of age at 3-month intervals until the rats were 12 months old.

Methods: Lenses of 15 rats were examined with both light and electron microscopes.

Results: At 12 weeks, opacity was observed in the perinuclear zone and the cortical intermediate layer. Liquefaction of the posterior subcapsular area and regression of cortical superficial fibers were also observed at this stage. Epithelial cells at the anterior polar area were multilayered. At 12 months, the lens recovered as a result of the regenerated lens fibers in the intermediate layer and the cortical superficial layer, although the opacity remained in the perinuclear zone. The multilayered cellular structure in the center of the epithelium returned to its original monolayer form. However, the equatorial epithelial cells became vacuolated and swollen with age, showing regression from the bow region.

Conclusions: These results suggest that the decrease of opacity in SCR rats is merely a temporary phenomenon that reflects the differentiating and metabolizing functions of the epithelial cells. With initiation of epithelial regression, the regeneration of the lens fibers ceased, suggesting that further decrease in opacity was no longer possible. **Jpn J Ophthalmol 1999;43:363–367**

Key Words: Lens epithelium, lens recovery, mature cataract, posterior cortical fibers, SCR rat.

Introduction

It is well known that during the development of a mature cataract, degeneration and liquefaction of the remaining lens fibers progresses, and the lens becomes liquefied, emulsified, membranous, and finally flattened, leading to a hypermature cataract. In several types of hereditary cataracts in mice[1–4] and rats,[5] it has been reported that the entire lens is atrophied after the development of a mature cataract because of the degeneration and liquefaction of the remaining lens fibers and posterior movement of the nucleus, similar to the pathological course observed in the human eye.

The SCR rat species, which was established by Shumiya and Nagase,[6] develops a mature cataract at 11 postnatal weeks at a 60% probability.[7] The morphological mechanism of the onset of this cataract is the extension of anterior sutural hypoplasia and liquefied anterior cortical fibers towards the posterior subcapsular region, which causes opacity.[8] In our long-term observation of this cataract for a period of 12 months, it was found that normal cortical fibers were regenerated and the lens recovered, leaving the opacity only in the perinuclear zone. Accordingly, the SCR cataract showed quite a different course of development than cataracts reported previously. In this study, we describe the morphological features of the SCR cataract.

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Materials and Methods

Fifteen SCR rats (aged 3, 6, or 12 months) that had already developed lens opacity were maintained and used in this study according to the ARVO resolution on animals and ophthalmic research. These rats were kept under a 12-hour light–dark cycle and were provided with food and water ad libitum. The animals were sacrificed with an intraperitoneal injection of sodium pentobarbital at a dose of about 40 mg/kg. The eyes were enucleated and immersed in 4% glutaraldehyde–0.1 mmol/L phosphate buffer for more than 3 days. During this fixation, the lenses were extracted and then washed with the same buffer solution, followed by postfixation with 1% OsO_4–0.1 mmol/L phosphate buffer overnight. Specimens were dehydrated with ascending ethanols, propylene oxide, and then embedded in Quetol 812. Specimens were stained with toluidine blue and observed by light microscopy. Ultrathin sections were double-stained with uranyl acetate and lead citrate and then observed with an electron microscope (HU-12A; Hitachi, Mito). Separately, some of the extracted lenses were immersed in a 3:1 ethanol/acetic acid solution for 3 days and then stored in 70% alcohol. These specimens were placed in water after staining with hematoxylin for several minutes, and the epithelium was peeled off under a dissecting microscope. After staining with hematoxylin again, the peeled epithelium was embedded in balsam and observed by light microscopy.

Results

At 12 weeks of age (immediately after the development of opacity started in the lens) the nuclear region remained transparent, and the liquefied area extends to the posterior subcapsular region. Whereas the lens fibers in the perinuclear zone and cortical intermediate layer separated or became swollen and developed opacity (Figure 1a). Although the cortical superficial layer was still transparent, the lens fibers were regressive back to the equator, even to the region adjacent to the posterior polar region (Figure 1b). In the specimens with epithelial elongation, the epithelium on the separated anterior suture had invaded the anterior cortex and was multilayered (Figure 1c). In the equatorial epithelium, a considerable number of acellular areas (Figure 1d) were observed.

In rats aged 3 months, the cortical superficial layer increased slightly in thickness toward the posterior pole. The bow structure showed a small number of nuclei with posterior displacement, but the bow configuration was free from any major changes (Figure 2a). In electron microscopic observation of the equatorial epithelial cells and adjacent lens fibers, no significant structural differences from the controls could be identified (Figure 2b).

In rats aged 6 months, the fibers in the cortical superficial layer were elongated about 150 μm toward the posterior polar side, and the tip of the lens fibers looked like a slender cone. The anterior cortical fibers had thickened by about 200 μm, resulting in the

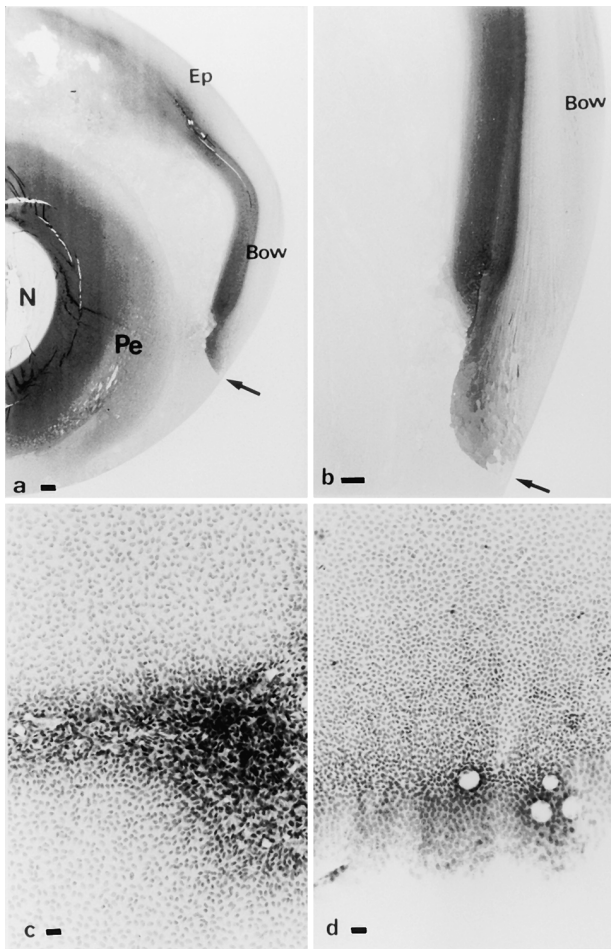

Figure 1. Photomicrographs of sagittal sections of SCR rat lens at 12 weeks of age. (**a**) Posterior elongation of lens fibers is interrupted just behind bow region (arrow). Ep: epithelium; N: nucleus; Pe: perinuclear region. (**b**) Higher magnification of interrupted lens fibers near bow region (arrow). Posterior ends of lens fibers adjacent to liquefied area appeared regressive. (**c**) Flat preparations of central area of lens epithelium. Epithelial cells are markedly proliferated at anterior pole. (**d**) Flat preparations of equatorial region of lens epithelium. Several circular acellularities are observed. Bars: (a) 100 μm; (b) 50 μm; (c and d) 20 μm.

reduction of opaque area. However, the perinuclear zone remained opaque, and the lens fibers were still collapsed (Figure 2c). In the magnification of the elongated cortical superficial layer, lens fibers newly differentiating from the equator appeared to elongate along the posterior capsule (Figure 2d). With recovery of the anterior suture that had been separated, multilayering of the epithelial cells decreased markedly (Figure 3a). The equatorial epithelial acellular areas were reduced in number, but had increased in diameter (Figure 3b). The arrangement of equatorial epithelial cells showed some irregularity in height, and the bow configuration was considerably irregular despite less posterior displacement (Figure 3c). A small volume of vacuoles could be seen by electron microscope in the capsular side of the cytoplasm of epithelial cells that appeared degenerative (Figure 3d).

In rats aged 12 months, the anterior cortical fibers increased, and the posterior cortical tip extended almost to the posterior pole (Figure 4a). Swelling of the lens fibers was frequently observed, and the arrangement of the fibers was somewhat irregular

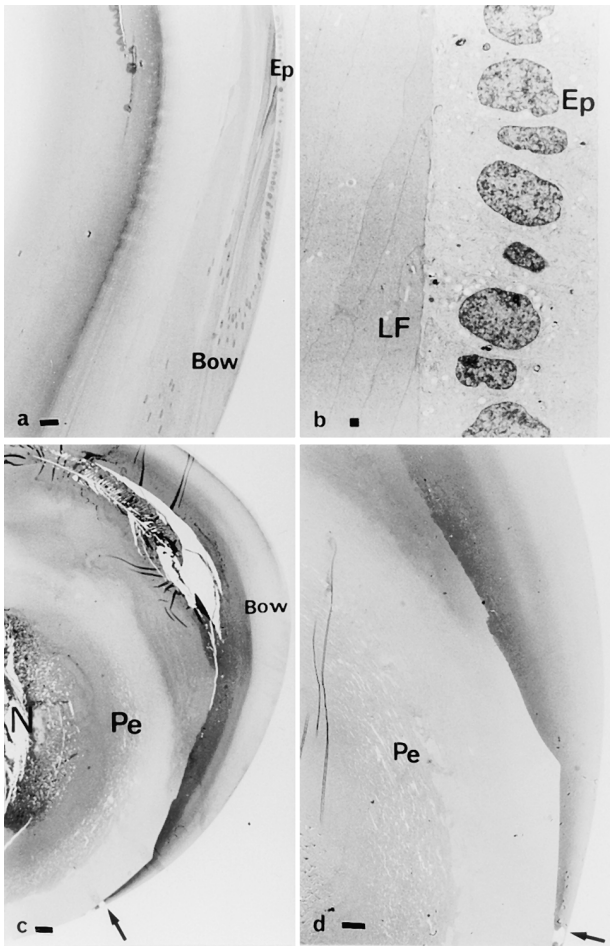

Figure 2. SCR rat lens at 12 weeks and 6 months of age. (**a**) Arrangement of epithelial cells (Ep) at equator and bow configuration appear relatively normal at 12 weeks, although some lens fibers are swollen. Ep: epithelium. (**b**) Higher magnification of same region as a. Cytoplasm of epithelial cells appears normal. LF: lens fibers. (**c**) Sagittal section of lens at 6 months of age. Superficial lens fibers are elongated toward posterior side (arrow). Opaque area in anterior cortex is reduced. Pe: perinuclear region. (**d**) Higher magnification of posterior ends of lens fibers in c (arrow). Ends are tapering. Pe: perinuclear region. Bars: (a) 20 μm; (b) 1 μm; (c) 100 μm; (d) 50 μm.

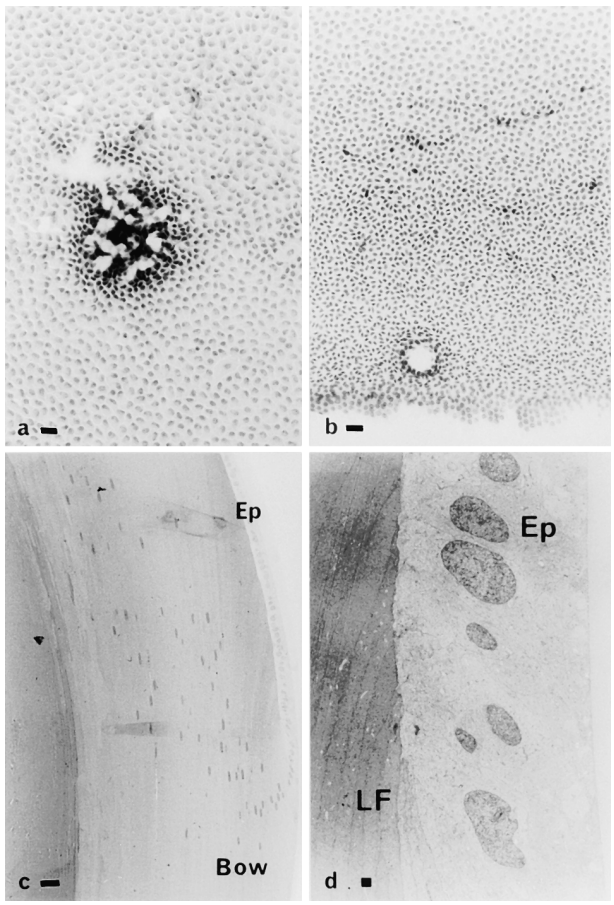

Figure 3. Flat preparations of central region of lens epithelium at 6 months of age. (**a**) Proliferated cells in polar region are reduced. (**b**) Flat preparations of equatorial region of epithelium at same age. Diameter of circular acellular area is enlarged. (**c**) Higher magnification of equatorial region in Figure 2c. Cytoplasm of epithelial cells (Ep) shows swelling. Bow configuration is somewhat irregular. (**d**) Electron micrograph of same area as c. Cytoplasm of epithelial cells is vacuolated and swollen. Bars: (a–c) 20 μm; (d) 1 μm.

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(Figure 4b). In the specimens with epithelial elongation, the multilayering of the epithelial cells present in the anterior pole disappeared (Figure 4c). The acellular area of the equatorial epithelial cells further increased in diameter and in number (Figure 4d). Regression of the equatorial epithelial cells started from the bow region, producing an epithelial deficiency between the bow structure and the epithelial cells (Figure 5a). Electron microscopic observation of the epithelial cells revealed slight swelling of the cytoplasm between dark cells, with cells suggesting degeneration intervening between them (Figure 5b).

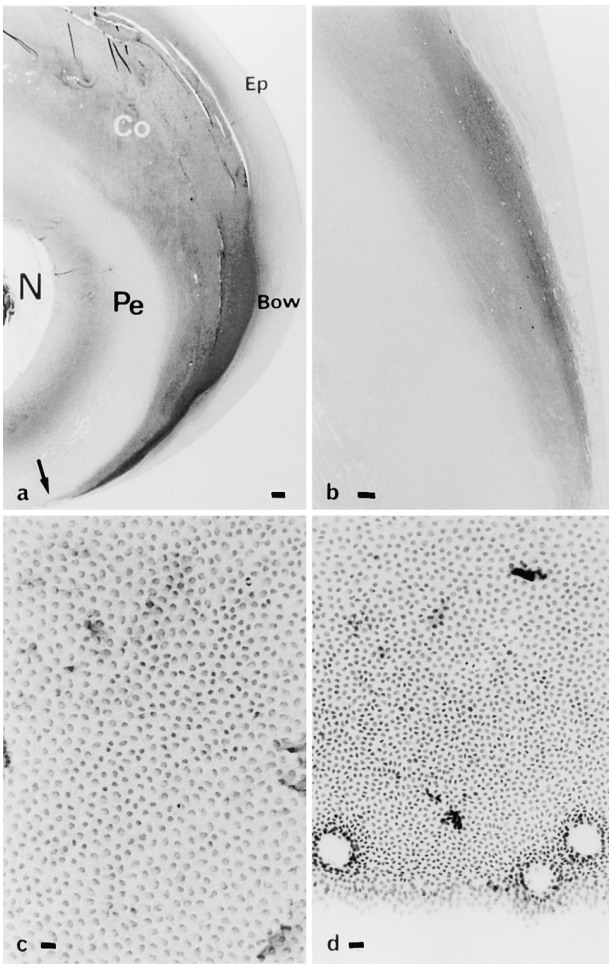

Figure 4. Photomicrographs of sagittal section of lens at 12 months of age. (**a**) Posterior ends of lens fibers extend slightly toward posterior pole (arrow). Size of normal cortical area (Co) is markedly increased. Ep: epithelium, N: nucleus, Pe: perinuclear region. (**b**) Higher magnification of posterior ends of elongating cortical fibers. Newly formed superficial fibers are somewhat shortened. (**c**) Flat preparations of central region of epithelium. Multilayered epithelial cells recover original monolayer arrangement. (**d**) Acellular areas of epithelial cells at lens equator are enlarged and more numerous. Bars: (a) 100 μm; (b–d) 20 μm.

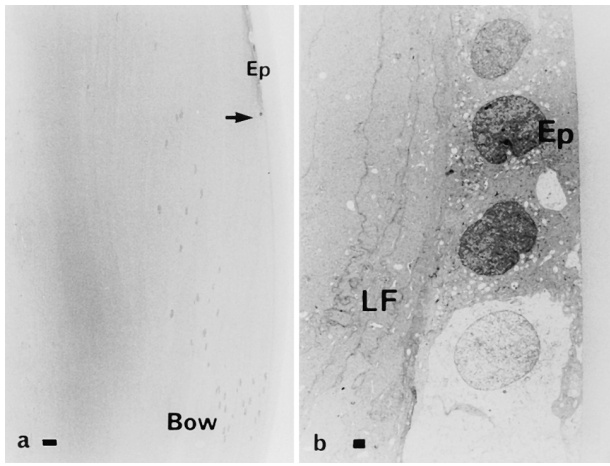

Figure 5. Higher magnification of equatorial region of Figure 4a. (**a**) Epithelial cells are fewer from bow region to site of arrow. (**b**) Electron micrograph of equatorial epithelial portion of Figure 5a. Epithelial cells (Ep) are often vacuolated and regressive. LF: lens fibers. Bars: (a) 20 μm; (b) 1 μm.

Discussion

SCR rats developed mature cataract at around 11 weeks of age, producing opacity from the perinuclear zone to the cortical intermediate layer.[8] At this time, the cortical superficial fibers showed atrophy and regressed to a region almost adjacent to the equator. Subsequently, cortical superficial fibers extended towards the posterior polar side, followed by recovery of the anterior cortical fibers, resulting in the presence of residual opacity only in the perinuclear zone. On the other hand, it was found that equatorial epithelial cells had degenerated, causing markedly reduced differentiation from epithelial cells to lens fibers. As a result, the recovery of the cataract ceased, and the cataract advanced to a hypermature stage. Figure 6 shows a schematic representation of the above-mentioned results.

In human senile cataract and hereditary cataract in the mouse[1–4] or rat,[5] once a mature cataract develops, it progresses to a hypermature cataract, completely failing to recover. The predictable outcome is that the development of a mature cataract results in an abnormality in the epithelial cells or lens fibers. These cells interact in an abnormal lesion, further advancing degeneration of the lens structure.

Pathologically, the major cause for the development of the type of cataract observed in the SCR

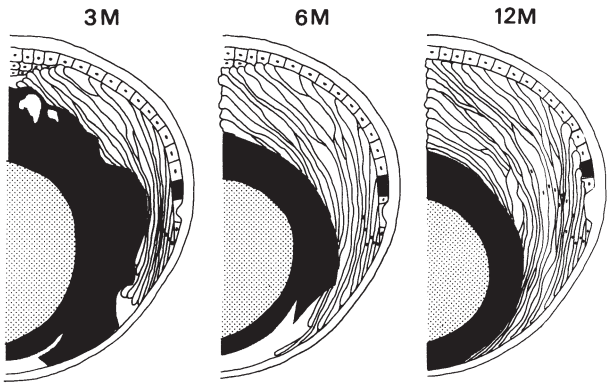

Figure 6. Schematic drawing showing reduction of opaque area of lens with increased age in SCR rat. M: month.

rats examined in our previous study[8] was separation and liquefaction of the anterior suture at around 8 weeks. The liquefaction ultimately extended as far as the posterior polar side, causing the posterior suture to separate. This resulted in the development of opacity in the perinuclear zone and intermediate zone. On the other hand, the separated part of the anterior suture was covered gradually by the proliferative epithelial cells. However, upon the completion of repair, these proliferative epithelial cells gradually decreased in number and were restored to their original monolayer form. This can be well understood from the arrangement of the epithelial cells in the center of the flat preparations of epithelium. This course of repair mimics that observed when injury is induced experimentally via the cornea on the anterior side of the lens with a needle.[9] It has been reported that the injured part becomes coated with proliferative epithelial cells and that invasion of the aqueous humor from the injured site ceases within 1 week.[10] Afterward, the number of epithelial cells growing on the injured site decreases gradually, leading to restoration of the original monolayer form.[11]

The relatively slight abnormality of the subepithelial anterior cortical superficial fibers is another notable point observed in SCR rats. This suggests that the cataract has a lesser secondary effect on the epithelial cells. However, once opacity develops in the perinuclear zone the condition is irreversible. It has been pointed out that the perinuclear zone shows a very sensitive reaction to the state of suture and liquefaction; opacity develops first in this area and extends towards the surrounding fiber cells, establishing a total cataract.

In brief, the mechanism of opacity development in the cataract of SCR rats consists of the development of anterior sutural separation and liquefaction, followed by extension towards the posterior polar side. As the separation of the anterior suture and swelling of the anterior cortical fibers were relatively mild, and the repair of epithelial cells was completed at an early phase, a temporary recovery of the cortical fibers was seen. However, resulting from the secondary effect of the opaque area, the epithelial cells seemed to degenerate again and a severe cataractous condition set in.

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