The Internalization of Posterior Subcapsular Cataracts (PSCs) in Royal College of Surgeons (RCS) Rats. II. The Inter-Relationship of Optical Quality and Structure as a Function of Age

Jer. R. Kuszak,1,2 Kristin J. Al-Ghoul,1 Layne A. Novak,1 Kurt L. Peterson,1 Kelly L. Herbert,1 Jacob G. Sivak3

Departments of 1Pathology and 2Ophthalmology, Rush-Presbyterian-St. Luke’s Medical Center, Chicago, IL; 3School of Optometry, University of Waterloo, Waterloo, Ontario, Canada

Purpose: The Royal College of Surgeons (RCS) rat is an animal model for human retinal degenerative disease and posterior subcapsular cataracts (PSCs). The purpose of this study was to correlate the structure and optical quality of RCS lenses with PSCs as a function of their internalization, with normal, non-cataractous, age-matched control lenses.

Methods: Correlative light (LM), scanning electron microscopic (SEM), three-dimensional computer assisted drawings (3D-CADs) and low power helium-neon laser scan analysis were used to examine the structure and function of lenses.

Results: The optical properties (average focal length variability; sharpness of focus) of RCS rat lenses are quantitatively compromised by PSCs. Correlative LM and SEM analysis of RCS lenses at various stages of PSC internalization (1.5, 3, 6, 9, 12 and 15 months of age), revealed that the sutures formed by additional fiber growth were progressively more abnormal. During PSC internalization, two to nine small suture branches were formed and arranged in modified line to multiple y configurations rather than the normal three branch y sutures. These temporal changes were also chronicled in animated 3D-CAD videos derived from lens reconstructions based on LM and SEM micrographs from the selected time points stated above. However, laser scan analysis also revealed that as the PSCs of RCS rat lenses were progressively internalized, there was a steady improvement in total sharpness of focus that reached normal levels by 12 months of age. The correlation of laser scan and structural data from specific regions of lenses revealed the following: 1. The abnormal posterior sutures of RCS rats with internalized PSCs effect a greater reduction in optical quality than normal posterior sutures of RCS rats with internalized PSCs effect a greater reduction in optical quality than normal posterior sutures of age-matched controls; 2. However, the resulting abnormal suture plane area was cumulatively similar to that of age-matched controls; 3. Thus, total optical quality was similar between RCS lenses with internalized PSCs and age-matched controls by 12 months of age.

Conclusions: The results of this study show that RCS lenses with internalized PSCs can appear grossly, and indeed optically perform, at levels comparable to aged lenses. These findings are consistent with clinical observations of spontaneous recovery from PSC. The results suggest that human PSCs that occur as a consequence of retinal degenerative disease could also be the result of abnormal posterior suture growth. If this is proven to be the case, such PSCs may have some capacity for repair or recovery thereby obviating their surgical removal.

Posterior subcapsular cataracts (PSCs) are a common complication of retinal degenerative disease [1,2]. While the removal of the PSCs improves vision, it can also hasten the ongoing degeneration of the retina [3]. Thus, the more preferred course of action for such PSCs would be treatment rather than surgical extraction. Unfortunately, an efficacious therapeutic intervention to effect recovery from this, or any other type of cataract, is not currently available.

The Royal College of Surgeons (RCS) rat is an established animal model for human retinal degenerative disease [4-10]. In the RCS rat, retinal degeneration occurring from 2-6 weeks after birth results in the formation of a PSC [11]. These PSCs are specifically characterized by abnormal fiber growth that results in the failure to form typical inverted posterior Y sutures in successive growth shells during the period of retinal degeneration. Instead, in successive shells, abnormally enlarged and more irregular posterior fiber ends turn up and away from the polar axis to form a posterior subcapsular plaque. However, after retinal degeneration is complete, approximately 75% of lenses with PSCs are said to recover from this cataract. Recovery is effected by additional fiber growth, internalizing the PSC [12]. However, the posterior sutures formed by these additional fibers are abnormal. Furthermore, it is not known how the recovered lenses perform optically.

If a cataractous lens can regain transparency with additional, less than normal lens growth, it would be useful to understand how this can be accomplished so that the efficacy of therapeutic intervention to ameliorate or prevent such cataracts could be quantifiably ascertained.

Therefore, the purpose of this study was to quantify the relationship between lens sutures and lens optics as a function of age during the internalization of, or recovery from, PSCs in RCS rats.

METHODS

A total of 68 Royal College of Surgeons (RCS) rats were used in this study. RCS rats with posterior subcapsular cataracts were examined by correlative low power helium-neon laser optical and structural analysis (light and scanning electron microscopy) at 1.5-2 (n=26), 3-4.5 (n=20), 5-6.5 (n=13), and 12-15 months (n=9) of age. Age matched Sprague-Dawley rats were used as controls (n=23 at 1.5-2 months of age; n=27 at 3-4.5 months of age; n=12 at 5-6.5 months of age; n=26 at...
12-15 months of age; total number control n=88). All animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Resolution for the use of animals in medical research.

**Optical Quality Analysis:** Within two minutes of sacrifice, eyes were carefully excised and the lens was removed from the eye. At this time the axial dimensions and gross appearance of lenses (anterior and posterior suture patterns) were recorded under a Zeiss (New York, NY) surgical dissecting microscope equipped with a Kodak Professional DCS 420 digital camera (Eastman Kodak Co., Rochester, NY). Within five minutes of sacrifice, lenses were placed in a special two-chambered cell made of glass and silicon rubber [13]. Both lens surfaces were bathed in a culture medium (25 ml) consisting of M199 (Gibco, Grand Island, NY) with Earle's salts and 8% fetal bovine serum. Previous work involving periodic measurements of medium osmolarity and pH indicated little or no change for the duration of the experiment. Each lens was suspended within the test cell on a beveled washer designed to support its equatorial rim. A mechanical linkage made it possible to rotate the washer (and lens) through an angle of 90°. The optical quality of lenses was then analyzed using a low power helium-neon laser scanner.

The laser scanner consists of a low-power (2 mW) helium-neon laser mounted on a computer-controlled X-Y table, and a television camera with a video frame digitizer [13]. A scanning laser beam is used, so that a number of beams pass at precise increments through different parts of the lens either on, adjacent to or completely off lens sutures, in order for the degree of lens spherical aberration to be determined and compared as a function of the above described distinct structural parameters.

Focal length (equivalent focal length; fl) was measured from the principal plane (intercept of incoming beam with exiting beam) to the intercept of the beam with the optical axis. Changes in this distance with beam position are influenced by the presence of coma and the degree of longitudinal spherical aberration, but the degree of spherical aberration is the dominant factor. In addition to measuring the refractive condition of the lens under study, the scanning laser system can measure the relative change in lens scatter (transparency) for each laser position. However, scatter measurements have proved to be more difficult to interpret than focal measures, and this study therefore concentrates on focal length results. Repeated measurements indicate instrument repeatability ±0.32% of focal length.

The growth and development of the lens can be compared to that of a tree, with new growth occurring peripherally and older material becoming concentrated toward its center throughout life. In fact, the focal length profiles produced by the laser scanning apparatus can be compared to a densitometry scan of a series of growth rings of a tree. In both cases, the resulting description will be very idiosyncratic and will reflect the influence of a variety of factors affecting growth.

Focal length results were analyzed both in terms of average focal length for 18 scanning positions and in terms of focal length variability (spherical aberration or sharpness of focus). Typically the suture branches in successive growth shells of rat lenses are oriented as an upright Y anteriorly, and an inverted Y posteriorly [14,15]. Ideally, it would have been of interest to scan lenses directly along and off of sutures, as in earlier work with rabbit, bovine and monkey lenses [16-18]. In this manner, a direct indication of suture effects on lens optical quality can be quantified. However, the relatively light weight of rat lenses makes it impossible to guarantee suture alignment relative to the laser beam while rotating the lens to provide scans of differing and known orientations. Therefore, in this study, lenses were only scanned along two directions (x and y) 90° apart from each other, and the resulting measures averaged for each lens. Thus each lens was scanned twice for a total of 36 scanning positions per lens (Figure 1) or a grand total of 5,652 objective measures for the complete study. Focal length variability (flv) is defined as the standard error of the mean of the lens focal length (mm) with inter-animal variance expressed as ±SEM [16].

**Structural Analysis:** Following laser scan analysis (within ten minutes of sacrifice), lenses were immediately placed into a chemical preservative (2.5% glutaraldehyde in 0.07 M sodium cacodylate, pH 7.2) for microscopic analysis [19]. Briefly, lenses were fixed at room temperature for five days with daily changes of fresh fixative. The seemingly excessive length of immersion fixation, necessitated by the high protein content of the lens and its lack of blood supply, does not adversely affect lens morphology [19,20]. After an overnight buffer wash, complete and intact suture patterns were dissected from all lenses as described previously [17]. After dissection, lenses or lens pieces were osmicated overnight in 1% aqueous OsO₄. Following an overnight buffer wash, the tissue water of the lens discs was removed by dehydration through a graded series of ethanol to absolute ethanol. The absolute ethanol was then replaced through a graded series of Freon 113/absolute ethanol to 100% Freon 113. Lens pieces were then critical point dried in Freon 13 in a Balzers CPD 020 (Balzers, Hudson, NH). Critical point dried lenses and/or pieces were secured onto aluminum stubs with conductive sil-

![Figure 1. Low power images of fixed control rat lenses as seen under a stereo-view dissecting microscope. The typical upright anterior Y suture pattern is readily apparent (A). Note that when a standard map of laser penetration points is overlain on such lenses, it can be seen that the 0-180° series of lasers will pass essentially directly along both the dorsal posterior (unseen) and ventral anterior suture branches (B). In contrast, the 90-270° series of lasers will pass essentially in between both the pair of dorsal anterior branches and the single ventral anterior branch, as well as between the single dorsal posterior branch (unseen) and pair of ventral posterior branches (unseen).](http://www.molvis.org/molvis/v5/p7)
ver paint. Specimens were mounted on their convex surfaces so that their concave surfaces were oriented at approximately 90° to the direction of the electron beam. All specimens were sputter coated with gold in vacuo. The specimens were examined in a JEOL JSM 35c scanning electron microscope (Peabody, MA) at 15 kV. Micrographs were taken with a Polaroid (Cambridge, MA) camera system at f11. SEM stereopair micrographs were taken at ±6°.

Suture patterns were derived from both light and SEM photomicrographs of lenses between 10 and 40x magnification. The ultrastructure of individual suture branches was examined at higher magnification (100-3,000x). Scaled three dimensional computer assisted drawings (3D-CADs) were made from suture patterns using a 3D-CAD computer program (TOPAS Professional; Crystal Graphics, Santa Clara, CA) as described previously. Briefly, by relating intact suture patterns as a function of radial location within (intra-lens sutural analysis) and between lenses of different ages (inter-lens sutural analysis), it is possible to reproduce an accurate reconstruction of 3D lens sutural anatomy as a function of development, growth and age (Figure 2) [17,18,21].

RESULTS

RCS Lens Structure As A Function of PSC Internalization: After the initial internalization of posterior subcapsular cataracts (PSCs), which occurs between 2-3 months of age [12], lenses were essentially transparent at all subsequent ages examined. However, their sutures were readily discerned as broad translucent lines under a dissecting microscope. The suture pattern of a representative 3.5 month old RCS rat lens as seen under a stereo dissecting microscope is shown in Figure 3A. Typically, at this age, rat lenses feature an inverted Y suture [14,15]. But it is apparent from Figure 3A that after the PSC was internalized by the overlaying of posterior segments of fibers, the ends of these successive fibers overlapped to form abnormal posterior suture patterns. In this instance, the resulting posterior suture pattern was small, centrally located, inverted Y suture with three secondary branches extending to...
confluence at the peripheral ends of two of the primary or main suture branches. The suture pattern as seen through a stereo dissecting microscope of a representative 15 month old RCS rat lens, dissected down to the axial dimensions of a 6 month old RCS rat lens, is shown in Figure 3B. While comparable to that seen at three months, this abnormal posterior suture pattern was slightly more irregular and complex. Once again this suture had a small, centrally located, inverted Y suture, but this time it featured three secondary branches extending to confluence at the peripheral ends of two of the primary or main suture branches and two tertiary sub-branches extending to confluence at the peripheral end of one of the secondary branches. Because the suture branches were slightly less discernible in dissected lenses, the patterns and ultrastructure of sutures from these lenses were further analyzed by scanning electron microscopy (SEM). The suture pattern of a representative 12 month old RCS rat dissected down to the axial dimensions of a three month old rat as seen by SEM is shown in Figure 4A. The two secondary branches extending to confluence at the peripheral end of one of three primary branches (box on Figure 4A) is shown at higher magnification in Figure 4B. At higher magnification all suture branches were seen to be the result of the overlap of even more en-

Figure 3. Low power images of RCS rat lenses as seen under a stereo-view dissecting microscope. A representative unfixed 3.5 month old RCS rat lens (A) and a fixed 15 month old RCS rat lens (B), dissected to the axial dimensions of a 6 month old RCS rat lens (intra and inter lens analysis). It is apparent that in both lenses the PSC was internalized by the overlaying of posterior segments of fibers to form abnormal posterior suture patterns. At 3.5 months of age, the resulting posterior suture pattern was an inordinately small, centrally located, inverted Y suture with three secondary branches extending to confluence at the peripheral ends of two of the primary or main suture branches. At 6 months of age, the abnormal posterior suture pattern was slightly more irregular and complex. This suture had an inordinately small, centrally located, inverted Y suture, with three secondary branches extending to confluence at the peripheral ends of two of the main suture branches and two tertiary sub-branches extending to confluence at the peripheral end of one of the secondary branches.

Figure 4. Series of scanning electron micrographs showing an abnormal posterior suture pattern from a 6 month old RCS rat lens. (A) By dissecting this lens to the axial dimensions of a 2.5 month old lens, a slightly atypical anterior Y suture pattern with a single sub-branch extending to confluence at a dorsal branch (box) is exposed. (B) This dorsal anterior branch and its sub-branch are shown to greater advantage at higher magnification. The cellular surface structure within the boxes shown in (B) are shown to greater advantage at higher magnification in (C) and (D). The ends of fibers are more enlarged than usual as they abut and overlap to form atypical anterior suture branches and sub-branches.

Figure 5. Creation of texture maps from tracings of scanning electron micrographs. (A) Higher magnification of scanning electron micrograph in Figure 4B. By tracing fiber groups from the equator to their sutural terminations (B) scale computer generated texture maps are made of representative growth shells. In this manner, lens suture patterns are analyzed as a function of age, and during posterior subcapsular cataract internalization as seen in Figure 6, Figure 7, Figure 8, and Figure 9.
larged and non-uniform fiber ends than normal (Figure 4B-D).

Using both light and SEM micrographs from recorded depths along the visual axis of both comparably aged and variably aged lenses, anterior and posterior suture patterns were recorded as a function of age. From these micrographs, tracings of fiber groups from the sutures to the equator (Figure 5) allowed for the recording of exact variations in fiber width, length and curvature in representative growth shells as a function of age (Figure 5 and Figure 6).

Representative anterior suture patterns are shown in Figure 6. At 3 months of age, irrespective of the fact that a PSC had formed from 2-6 weeks of age [11], a relatively normal Y suture had formed anteriorly during the same time period (Figure 6A). This suture was not deemed normal for the following reasons: 1. Although it had the typical three branches arranged in a Y suture pattern, the branches were considerably shorter than those seen in age matched control lenses; 2. The branches were not equal in length; and 3. They were not aligned precisely 120° apart from one another. After 3 months of age,
subsequent anterior suture patterns formed were even more irregular. These sutures, consistently featured shorter than normal branches, with one of the upper suture branches progressively overlain by two smaller branches extending to confluence at the confluence of the two other branches (Figure 6B). By 6 months of age, these four branches of variable length had formed an offset “t” or “cross” suture pattern (Figure 6C). By 9 months of age, the four branches were separated by a central vertical branch (Figure 6D). Alternatively, this suture pattern could be described as an upright Y suture and an inverted Y suture that shared a single vertical branch. The subsequent sutures formed from 12 (Figure 6E) to 15 months of age (Figure 6F) had comparable patterns that were variably offset in successive growth shells and branches that were more variable in length.

Representative posterior suture patterns are shown in Figure 7. At 2 months of age, a very irregular Y suture had formed over the PSC (Figure 7A). First, this suture was relatively

Figure 8. Scale 3D-CAD animation showing the abnormal anterior and posterior suture planes formed during PSC internalization. Over the course of one year following retinal degeneration, continuous lens growth in RCS rats results in the overlap of atypical suture patterns in successive growth shells. Thus, multiple, small, triangular and rhombic, abnormal anterior and posterior suture planes are produced and aligned directly along the visual axis.

A quicktime movie can be viewed online at the following URL: http://www.molvis.org/molvis/v5/p7/kuszak-fig8.html/. A representative frame from the movie is shown to the left.

Figure 9. Scale 3D-CADs of abnormal anterior and posterior suture planes formed as a function of age during internalization of PSC. The evolution of small, triangular and rhombic suture planes produced by the overlap of the atypical suture branches in successive growth shells is shown at (A) 2 months, (B) 3 months, (C) 6 months, (D) 9 months, (E) 12 months, and (F) 15 months of age. Comparing this figure to Figure 6 and Figure 7 illustrates that the overlaying of abnormal suture patterns from successive growth shells produces abnormal suture planes. The temporal nature of suture malformation during PSC internalization is shown to greatest advantage in animated form in Figure 8.
upright, a characteristic of anterior lens sutures of rats, rather than inverted as is typical of normal rat posterior sutures. Second, as seen in anterior sutures, the three unusually short branches were also unequal in length. In addition, their angular placement relative to one another was also atypically unequal. In fact, this suture pattern was not unlike a crooked vertical line suture with two small secondary branches extending to confluence at the upper end of the main branch. By 3 months of age, subsequent posterior suture patterns were even more irregular (Figure 7B). These sutures consistently featured one of the upper suture branches being overlain by two even shorter branches extending to confluence at a primary branch. By 6 months of age, subsequent sutures had a similar pattern, but with different length branches (Figure 7C). By 9 months of age, a total of six branches combined to form an irregular combination of an upright Y suture and an inverted Y suture that shared a single vertical branch as seen anteriorly (Figure 7D). From 12 (Figure 7E) to 15 months of age (Figure 7F) the subsequent posterior sutures formed had comparable patterns but with additional secondary branches that had

Figure 10. Scale 3D-CADs comparing the abnormal anterior and posterior suture planes formed during internalization of PSC and the normal anterior and posterior suture planes formed during lens growth. In a 15 month old RCS rat lens viewed at a slight angle to the optical axis (A) and along the equatorial plane (B) it can be seen that the complex and abnormal set of suture planes do not extend into the lens periphery. In contrast, in a 15 month old control rat lens viewed at a slight angle to the optical axis (C) and along the equatorial plane (D) it can be seen that the simple normal suture planes of age-matched controls extended farther into the lens periphery.
extended to confluence at the peripheral ends of main branches. By 15 months as many as nine small branches combined to create suture patterns that can be accurately described as a combination of an upright Y suture and an inverted Y suture that shared a single vertical branch, and an offset upright Y suture that shared a single upper branch of the combined upright Y suture, and an offset inverted suture that shared a single lower branch of the combined inverted suture. The overlaying of progressively more abnormal anterior and posterior suture patterns to internalize posterior subcapsular cataracts in RCS rats as a function of age can be more readily appreciated by viewing the process temporally in the animation seen in Figure 8.

By aligning the anterior and posterior suture patterns of RCS rat lenses as a function of age along the polar axis, the continuous and progressively more irregular suture planes that had existed in situ were reconstructed in scaled 3D-CADs (Figure 8, Figure 9, and Figure 10). By this method it was readily apparent that numerous, small, variably polygonal (triangular and rhombic), and continuous suture planes had been aligned directly along the visual axis extending from the embryonic nucleus anteriorly and from the PSC plaque posteriorly to, respectively, the anterior and posterior surfaces (Figure 9A,B).

By comparison, in aged matched control lenses, the sutures were consistently seen anteriorly to be upright Y suture patterns composed of three branches of equal length and positioned at essentially 120° to one another, while posteriorly the same type of suture patterns were seen except in the inverted

Figure 11. Plots of laser scan analysis through RCS rat lenses. Consider the standard map of laser penetration points directed into a rat lens by our low power helium-neon laser scan analysis as shown previously in Figure 1B. If the lens is turned 90° on its equatorial axis, the variable focal lengths of the eighteen laser beams can be represented as they are reflected or refracted by the lens. The vertical axis indicates laser beam position (mm) from the optical center (0, 0) of the lens. The horizontal axis indicates equivalent focal length (mm). Plus (+) signs indicate the focal lengths for each incident laser beam as a function of distance from the optical center.

RCS rats at 1.5, 6, and 12 month old had a fully formed PSC, a PSC with 4.5 months of internalization by abnormal fiber growth, and a PSC with 10.5 months of internalization by abnormal fiber growth, respectively. Note that while our laser scan analysis routinely directs a laser beams at eighteen locations on a lens per pass (vertical axis), the PSC plaque in 1.5 months old RCS lenses internally reflected almost half of the beams on average. In contrast, internalization of the PSC plaque by abnormal fiber growth effectively produced a larger lens with an added posterior component. This new posterior component allowed the lens to recover the ability to refract all eighteen laser beams.

Figure 12. Plots of laser scan analysis through 1.5, 6, and 12 month old control rats. Consider the standard map of laser penetration points directed into a rat lens by our low power helium-neon laser scan analysis as shown previously in Figure 1B. If the lens is turned 90° on its equatorial axis, the variable focal lengths of the eighteen laser beams can be represented as they are reflected or refracted by the lens. The vertical axis indicates laser beam position (mm) from the optical center (0, 0) of the lens. The horizontal axis indicates equivalent focal length (mm). Plus (+) signs indicate the focal lengths for each incident laser beam as a function of distance from the optical center.

In the rat lens, optics are maximal at 6 months of age. By 12 months the negative effects of aging are apparent (see Table 1, Table 2, and Table 3).
configuration. Thus, by aligning these anterior and posterior suture patterns along the polar axis and within scaled 3D-CADs of control rat lenses as a function of age, reconstructions of the continuous, triangular suture planes that had existed in situ were produced and are shown in Figure 10C,D. By this method it was readily apparent that normally six, equal and continuous triangular suture planes were produced and aligned directly along the visual axis, extending from the embryonic nucleus anteriorly and posteriorly to, respectively, the anterior and posterior surfaces.

RCS Lens Optical Quality As A Function of PSC Internalization: Representative laser scan plots of RCS rat lenses at 1.5, 6 and 12 months of age are shown in Figure 11 while representative age-matched controls are shown in Figure 12. The negative influence of the PSC plaque on lens focusing was severe at 1.5 months of age. Of the eighteen laser beams directed at the lens, only ten were refracted by the lens to permit quantitative analysis of average focal length variability (average flv). The other eight were internally reflected by the PSC plaque. Although the average focal length (average fl) for RCS lenses was significantly reduced (p<0.03) in comparison to control lenses at all ages, there was no significant difference between RCS and control lenses in the expected age-related increase in average fl as a result of continuous lens growth (Table 1). The average fl of 6 month old RCS rat lenses was an exception, having decreased from that of 1.5 month old RCS rat lenses. This decreased average fl was a direct consequence of the fact that the 1.5 month old RCS had a PSC (RCS PSCs form from 2-6 weeks after birth [11]) while the 6 month old RCS rat lens had a PSC that had been internalized by 4.5 months of additional fiber growth (the onset of RCS PSC internalization occurs typically by 2 months of age [12]). Over the course of one year there was a 16.9% increase in average fl in RCS rat lenses while control lenses had a 14.4% increase in average fl during the same time period.

A more accurate assessment of lens optical quality is average focal length variability (spherical aberration measured in millimeters; average flv). At 1.5, 3 and 6 months of age the optical quality of control lenses was significantly better (p<0.01) than that of comparably aged RCS rats (Table 2). Overall total flv in control lenses increased slightly from 1.5 and 3 months of age, decreased by half by 6 months of age, and then increased again by 12 months of age. By comparison in RCS lenses, total flv decreased sharply from 1.5 to 3 month old rats, decreased less precipitously in 6 month and 12 month old rats. These results demonstrate that while total flv was 3 times greater in RCS rat lenses with PSC than in age matched non-cataractous control lenses, as addition of successive growth shells internalized the PSC of RCS lenses, the cumulative optical quality of these lenses became nearly equal to that of age-matched controls.

However, our laser scan optical analysis permits a comparative analysis of spherical aberration from specifically defined regions of lenses. In this manner, the effect of PSC and/or abnormal sutures in RCS lenses can be ascertained and compared directly to continued growth in control lenses. There-

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Average Focal Length (mm) RCS</th>
<th>Average Focal Length (mm) Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>4.53±0.24</td>
<td>5.33±0.03</td>
</tr>
<tr>
<td>3</td>
<td>4.85±0.09</td>
<td>5.88±0.04</td>
</tr>
<tr>
<td>6</td>
<td>4.74±0.14</td>
<td>6.00±0.06</td>
</tr>
<tr>
<td>12</td>
<td>5.30±0.11</td>
<td>6.23±0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Average Focal Length Variability (mm) RCS</th>
<th>Average Focal Length Variability (mm) Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.67±0.08</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.26±0.03</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>6</td>
<td>0.20±0.03</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>12</td>
<td>0.17±0.02</td>
<td>0.18±0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Average Focal Length Variability (mm) Central Region</th>
<th>Average Focal Length Variability (mm) Peripheral Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.86±0.11</td>
<td>0.48±0.05</td>
</tr>
<tr>
<td>3</td>
<td>0.43±0.05</td>
<td>0.23±0.06</td>
</tr>
<tr>
<td>6</td>
<td>0.34±0.03</td>
<td>0.23±0.05</td>
</tr>
<tr>
<td>12</td>
<td>0.33±0.06</td>
<td>0.11±0.01</td>
</tr>
</tbody>
</table>
fore, the flv data was analyzed to determine average flv separately in the central and peripheral regions of lenses. By definition, the inner sixteen laser beams transmitted by two scans (8 and 8; see Figure 1, Figure 10 and the animation in Figure 8) passed through that area of control lenses where suture branches came to confluence, or alternately through the RCS PSC. In a similar manner, by definition, the outer twenty laser beams transmitted by two scans (10 and 10; see Figure 1, Figure 10 and Animation Figure 8) passed through that area of control lenses where suture branches were absent, or alternately through the area of RCS rat lenses without PSCs. When these measures were compared the optical quality of the peripheral region of all lenses was superior to that of the central region. More importantly, the optical quality of the central region of control lenses was significantly superior (p<0.01) to that of RCS lenses at all ages (Table 3). The central region average flv of control lenses was essentially identical from 1.5-6 months of age. It increased as a function of age from 6 to 12 months. By comparison, peripheral region average flv in control lenses was markedly less at 1.5 months of age, increased slightly by 3 months of age, decreased considerably by 6 months of age and then increased sharply by 12 months of age. In contrast, central region average flv of RCS lenses was 0.86 mm (±0.11) at 1.5 months of age, decreased sharply to 0.43 (±0.05) by 3 months, and then decreased to, and was maintained at, 0.34 (±0.03) in 6 and 12 month old rats. By comparison, peripheral region average flv in RCS lenses was greatest at 1.5 month of age. It decreased and was maintained by half through 6 months of age, and finally decreased even further by 12 months of age. These results demonstrate the following: both central and peripheral region average flv was 4 times greater in RCS rat lenses with PSC than in age matched non-cataractous control lenses. However, as addition of successive growth shells internalized the PSC of RCS lenses, the optical quality of the peripheral region of these lenses actually became superior to that of age-matched non-cataractous lenses.

**DISCUSSION**

In this study we have quantifiably demonstrated a direct relationship between defined abnormal lens structure within the circumscribed area of a posterior subcapsular cataract (PSC) and the resulting significant compromise in optical quality. Typically the posterior ends of rat lens fibers overlap to form three suture branches of equal length that are arranged in the form of an inverted Y [14,15]. In contrast, the PSCs of RCS rats are specifically the result of the posterior ends of lens fibers failing to form inverted Y sutures as the retina of these animals degenerate from 2 to 6 weeks after birth [11]. During this period, the abnormally enlarged and more irregular posterior ends of fibers turn up and away from the polar axis to form a central posterior cataractous plaque. Our correlative structure/function analysis revealed that the PSC plaque in RCS rat lenses internally reflected, on average, almost half of the laser beams used to measure spherical aberration. As a result, average focal length variability (flv) of these lenses was as much as 5x greater than that of age matched controls (Table 2 and Table 3).

This study also established a direct, quantifiable, relationship between moderately abnormal lens sutures of RCS lenses with internalized PSCs, and a significant cumulative improvement in the optical quality of these lenses as a function of growth and/or recovery. Both the anterior and posterior suture patterns of RCS lenses with internalized PSCs featured more numerous and very short suture branches in comparison to age matched controls. As a result the suture planes of these lenses, formed by the overlaying of suture patterns in successive growth shells, are individually smaller than in aged matched controls (Figure 9 and Figure 10). It is important to recognize that suture planes, not suture branches, exert a quantifiable negative influence on lens optical quality. Therefore, as predicted by previous studies [16-18], the central region of RCS rat lenses, that region that contained both an internalized PSC and greater suture plane area owing to abnormal suture formation following PSC internalization, had significantly greater average focal length variability than in normal age-matched controls. However, the peripheral region of RCS rat lenses, that region that was both devoid of an internalized PSC and had less suture plane area owing to abnormal suture formation following PSC internalization, had significantly less average focal length variability than in normal age-matched controls. These results clearly show that internalization of, or recovery from, PSCs in RCS rat lenses is accomplished with the re-establishment of a close semblance of normal lens structure, that in turn effects improved optical function. These findings suggest that if PSCs formed as a consequence of other disease processes (e. g. steroid use, vitrectomy, and perhaps even diabetes) have similar structural compromise, than all of these pathological lenses may have the inherent capacity to recover, thereby obviating the need for their removal. Indeed, spontaneous recovery from some types of human PSCs has been noted clinically [22-25].

In fact, these results further suggest that if the factors and local environmental conditions within the eye required for continued normal lens growth are elucidated, then therapeutic intervention to prevent either further structural compromise in the formation of PSCs, or reversal and/or recovery from this type of cataract, might be a reasonable future option to surgery. The results of this study also draw attention to the inter-relationship between lens, retinal, and vitreal homeostasis. It has been proposed that the degeneration of the retina from 2-6 weeks post-natal in the RCS rat effects the release of lipid peroxidase products into the vitreous [5,7]. This in turn compromises the normal growth of lens fibers and results in the production of the PSC. However, it has also been proposed that the degeneration of the retina could preclude the release of essential factors produced by the retina into the vitreous [26]. In this study, long after retinal degeneration, only a semblance of normal lens growth is effected. All of the above argue that the inter-relationship between the lens, retina and vitreous needs further study.

In summary, we have found that internalization of PSCs in RCS rats while not accomplished by normal lens growth, is effected by a close semblance of normal lens growth. The relationship between this less than normal structural recovery and lens optical function can be quantified. These results sug-
gest that the lens may be capable of recovering from posterior subcapsular cataractogenesis.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Khailash Bhuyan of the College of Physicians and Surgeons, Columbia University, New York, N.Y. and the Alcon Laboratories Inc., Fort Worth, TX for providing the Royal College of Surgeons rats used in this study. This work supported by grants from the NIH-NEI to JRK (EY-06642) and the National Science and Engineering Council of Canada to JGS and the Louise C. Norton Trust, Chicago, IL.

REFERENCES