Studies on human cataracts

III. Structural elements in nuclear cataracts and their contribution to the turbidity

Frederick A. Bettelheim, Ernest L. Siew, and Leo T. Chylack, Jr.*

Gross correlation coefficient between light-scattering intensity and the optical parameters obtained for 48 sections of eight lenses with nuclear cataracts were evaluated. On the basis of these and other data in the literature, the structural elements within the lens fiber are identified. These give rise to the optical parameters. It is proposed that three processes contribute to nuclear cataractogenesis: (1) syneretic process, (2) increase in the concentration but not in the size of protein aggregates, and (3) association (entanglement) between aggregates and optically anisotropic cytoskeleton or membrane components that leads to a decrease in structural birefringence.

Key words: aggregation, correlation coefficient, human lens, light scattering, nuclear cataract, syneresis

One of the major interests in studying nuclear cataracts was to see how the optical parameters obtained from light scattering could describe and classify nuclear cataracts and how this internal description agreed with the clinical description and classification. Besides this interest there is a second one, not less important: to establish what kinds of structures give rise to these optical parameters and how each of these structural elements in its turn contributes to the opacification.

Methods

Optical parameters of light scattering were evaluated with the theory of random density and orientation fluctuations. The relationship between the intensity of the scattered light and the optical parameters themselves were established by using least-squares linear-regression curves. To measure the degree of association between the random variables, the correlation coefficient, r, was calculated, as follows:

\[
 r = \frac{\sum_{i=1}^{N} x_i y_i/N - \bar{x} \bar{y}}{\sqrt{\sum_{i=1}^{N} x_i^2/N - \bar{x}^2}} \quad (1)
\]

where \(x\) and \(y\) are the independent and dependent variables, \(N\) is the number of samples (i.e., the number of section within one lens), \(\bar{x}\) and \(\bar{y}\) are mean values of the random variables, and \(\sigma_x, \sigma_y\) are the square roots of the variances, such as

\[
\sigma_x^2 = \frac{\sum_{i=1}^{N} x_i^2}{N} - \bar{x}^2 \quad (2)
\]

The statistical significance of each correlation...
coefficient was established by the standard Student's t test. All these calculations were performed on a Burroughs B 6700 computer using Minitab II program of the Pennsylvania State University.

Results and discussions

From the data presented in Part II, some general trends are observable in regard to the relationships between the structural parameters, $a_1$, $a_2$, and $A_1$—the size, separation, and concentration of the protein aggregates.

1. Where both the size of scattering units, $a_1$, and their volume fraction increased compared to the immediate spatial environment, the separation distance of the particles decreased. This happened in lens 73.

2. In cases where the size of the scattering unit was relatively constant throughout the lens, as the volume fraction of the scattering unit decreased the interparticle separation increased (lenses 80, 12, 13, and 01).

3. As both the size of the scattering unit and their separation decreased, the volume fraction of the scattering unit increased, and vice versa (lenses 67, 98 (over most of the lens), and parts of 72).

An interesting correlation was found between scattering intensity and the volume fraction of the dense scattering units. In many cases the scattering unit size was the same (such as lenses 01, 67, 12, 80, 73, 12), i.e., between 250 and 300 nm; in these cases the volume fraction of the scattering units varied across the lens.

<table>
<thead>
<tr>
<th>Lens no.</th>
<th>Intensity of scattered light at max.</th>
<th>Volume % where I is max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>850</td>
<td>36</td>
</tr>
<tr>
<td>80</td>
<td>670</td>
<td>24</td>
</tr>
<tr>
<td>73</td>
<td>850</td>
<td>36</td>
</tr>
<tr>
<td>12</td>
<td>530</td>
<td>25</td>
</tr>
<tr>
<td>01</td>
<td>900</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>400</td>
<td>16</td>
</tr>
</tbody>
</table>

Correlation $r^2 = 79.0\%$

A correlation existed between the turbidity when it was at a maximum and the volume fraction of the scattering unit at the same location. Thus for these cases it seems that the increased turbidity in the cataract formation was due not to the increase in the size of the aggregates but to the increase in the volume fraction of the aggregates.

For the remaining two cases there did not seem to be a simple correlation between aggregate size or the volume fraction and the magnitude of turbidity. In lens 98 the size of the dense aggregates varied between 600 and 270 nm, and the volume percent in general followed an opposing trend between 30% and 47%. When the size of aggregate was large, its volume fraction was low, and vice versa (sort of a canceling effect). In lens 98 the main cause of the scattering was the amplitude of fluctuation, $\eta^2$.

For lens 72, the size of the aggregate varied between 270 and 550 nm; the volume fraction followed a different trend, neither parallel to nor opposing that of the aggregate size, so that in this case too most of the turbidity due to density fluctuations can be correlated to the amplitude fluctuation $\eta^2$; however, the volume fraction and interparticle separation also played a decisive role.

Observations regarding the relationships of eight nuclear cataract lenses. On the basis of the primary data, especially the $I_+$, the scattering intensity in which both the primary beam and the scattered beam were parallel and which was usually 100 to 1000 times stronger than the depolarized scattering, $I_-$, one can divide the eight nuclear cataractous lenses into two groups. Group I had one broad maximum in the intensity of the scattered light as one moved across the lens; the maximum usually was near the center of the nucleus. Group II includes lenses that exhibited two maxima in the scattered light intensity as if the most opaque part of the cataract would form a ring surrounding the center of the nucleus.

Lenses 98, 80, 73, and 01 belong to group I, and lenses 72, 67, 12, and 13 belong to group II.

For group I an interesting correlation was found. If one selects the intensity of the scattered light at the location in each lens where it is the maximum and one correlates this...
with the density amplitude factor of each lens at the same location, a correlation coefficient of \( r^2 = 99.6\% \) is found. This implies that in nuclear cataracts in which the center of the lens is the most opaque, one can account for the densest cataract formation in each lens simply by the refractive index changes due to the density of the particles. Such simple correlation would imply that a synergetic process is the major contributor to cataractogenesis in these lenses. This is the highest gross correlation coefficient we found among our parameters and scattering intensity data.

No such correlation was found in the lenses of group II, in which the cataract seemed to have a ring character (\( r^2 = 17.3\% \)).

In order to establish how the different structural parameters affect the turbidity of the lens, the gross correlation coefficients were calculated. The gross correlation shows the relationship between the dependent variable (i.e., scattered light intensity at a certain angle) and an independent variable (the size of the protein aggregate), including the effects of other independent variables on the latter (how, for example, syneresis, the change in the amplitude measure, or refractive index influences the size of the aggregates). Since we were looking for trends only, it was not deemed necessary to calculate partial coefficients (first to seventh order) in which the effects of one to seven independent variables on the first independent variable would have been progressively removed.

The square of the gross correlation coefficient explains what percentage of the dependent variable is explained by the independent variable. For example in lens 98, \( r_{12}^2 \) is 80.9, which means that 80.9% of the intensity of the scattered light in the I, mode is accounted for within the cataractous lens 98.

Within one lens we were working with small numbers of thin sections (five to seven). In order that the correlation should be significant on the 95% level, the correlation coefficient squared had to be relatively high, as required by the t test. Correspondingly, when we investigated the trend for the total

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Table I. Gross squared correlation coefficients, \( r_{ij}^2 \), in percent between scattered light intensity and structural parameters for eight individual cataractous lenses and for the combined total

<table>
<thead>
<tr>
<th>Lens No.</th>
<th>( r_{1,2}^2 )</th>
<th>( r_{1,2}^2 )</th>
<th>( r_{1,4}^2 )</th>
<th>( r_{1,2}^2 )</th>
<th>( r_{1,4}^2 )</th>
<th>( r_{1,2}^2 )</th>
<th>( r_{1,4}^2 )</th>
<th>( r_{1,2}^2 )</th>
<th>( r_{1,4}^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>89.9*</td>
<td>23.0</td>
<td>44.2*</td>
<td>69.2*</td>
<td>42.0f</td>
<td>30.0</td>
<td>89.6*</td>
<td>37.9</td>
<td>45.5f</td>
</tr>
<tr>
<td>80</td>
<td>86.9*</td>
<td>61.3*</td>
<td>29.1</td>
<td>91.9*</td>
<td>81.2*</td>
<td>10.2</td>
<td>33.4</td>
<td>40.2</td>
<td>6.6</td>
</tr>
<tr>
<td>73</td>
<td>14.6</td>
<td>12.1</td>
<td>22.8</td>
<td>0.5</td>
<td>10.6</td>
<td>7.3</td>
<td>74.3*</td>
<td>2.0</td>
<td>47.9</td>
</tr>
<tr>
<td>01</td>
<td>67.9t</td>
<td>56.1</td>
<td>4.2</td>
<td>88.4*</td>
<td>65.0f</td>
<td>45.0</td>
<td>0.6</td>
<td>53.6</td>
<td>0.1</td>
</tr>
<tr>
<td>72</td>
<td>44.5f</td>
<td>69.7*</td>
<td>38.5</td>
<td>62.4*</td>
<td>65.9*</td>
<td>36.4</td>
<td>74.2*</td>
<td>59.2f</td>
<td>5.5</td>
</tr>
<tr>
<td>67</td>
<td>43.4</td>
<td>28.1</td>
<td>14.0</td>
<td>1.6</td>
<td>11.8</td>
<td>0.0</td>
<td>22.0</td>
<td>7.3</td>
<td>17.7</td>
</tr>
<tr>
<td>13</td>
<td>51.3</td>
<td>20.6</td>
<td>9.8</td>
<td>12.0</td>
<td>84.7*</td>
<td>37.0</td>
<td>30.0</td>
<td>37.3</td>
<td>14.7</td>
</tr>
<tr>
<td>12</td>
<td>49.6f</td>
<td>10.0</td>
<td>1.9</td>
<td>24.0</td>
<td>4.1</td>
<td>12.7</td>
<td>0.3</td>
<td>36.6</td>
<td>63.7*</td>
</tr>
<tr>
<td>Total</td>
<td>42.1*</td>
<td>13.8*</td>
<td>2.0</td>
<td>34.7*</td>
<td>30.5*</td>
<td>33.8*</td>
<td>30.0*</td>
<td>6.0</td>
<td>14.6*</td>
</tr>
</tbody>
</table>

Subscript numbers of the correlation coefficients refer to the following:

1 = \( L_1 \), intensity of scattered light when both polarizer and analyzer are aligned parallel to each other at 10-degree scattering angle.

2 = \( \eta_1 \), measure of the amplitude of density fluctuations describing the average size (nm) of the protein aggregates.

3 = \( a_s \), second correlation distance of density fluctuations describing the average separations in nm between protein aggregates.

4 = \( a_t \), volume fraction (concentration) of the protein aggregates.

5 = \( \eta_2 \), second correlation distance of density fluctuations describing the average separations in nm between protein aggregates.

6 = \( b_1 \), intensity of scattered light when polarizer and analyzer are set 90 degrees apart at 10-degree scattering angle.

7 = \( a_1 \), correlation distance of the density fluctuations describing the average separations in nm between protein aggregates.

8 = \( a_2 \), measure of the amplitude of density fluctuation (the mean squared deviations from the average refractive index).

9 = \( b_2 \), volume fraction (concentration of the optically anisotropic particles).

10 = \( \eta_3 \), second correlation distance of orientation fluctuations describing the average separation (nm) between the optically anisotropic particles.

11 = \( \eta_4^2 / b_3^2 \), dissymmetry of light scattered in the I, mode (the ratio between the intensity scattered at 10- and 30-degree scattering angles).

*Statistically significant at the 95% level.

fStatistically significant at the 90% level.
of eight lenses studied (48 thin sections), the criterion for statistical significance required a much smaller correlation coefficient. (Numbers underlined with solid line in Table I.)

In the calculation of correlation coefficients the assumption was made that the correlations have linear regressions. Although it is true that the relationship between I,, and 17* (1, 2) and 1+ and 82 (6, 7) is linear according to the theory, the theoretical relationships between the scattered intensities and other structural parameters are complex and non-linear. Therefore, the assumption that even these correlations have linear regressions involves an oversimplification. The true relationship for example between I,, and a3 (1, 3) is the area under a Gaussian curve upon which a sinusoidal fluctuation is superimposed. To approximate this relationship with a linear relationship is akin to smoothing out the seasonal fluctuations of a stock on the market and predict its future on the basis of past performance. Since in this study we are looking for general trends rather than future predictions, the oversimplification may not be disastrous. Furthermore, these arguments are used for comparison only—how the structural parameters correlate with the light scattering in one lens compared to a second lens or compared to the total number of lenses studied.

In order to find some interpretation for the numbers in Table I it must be kept in mind that (1) the assumption of linear relationship between the dependent and independent variables is an oversimplification and (2) we are dealing with gross correlation coefficients in which the effect of the other independent variables on the independent variable under scrutiny is not removed. This means that one should not expect that the sum of gross correlation coefficients of one dependent variable such as r,,  + r,, . . . + r,, should add up to 100%. With this in mind, first, one should take a look at the gross correlation coefficient of the total eight lenses. We find that almost all the correlations are statistically significant at the 95% level. One could state that the scattered light intensity in the I, mode is influenced most by the p4 parameter that is, the amplitude of the density fluctuation alone can explain 42.1% (r,,) of the intensity of the scattered light. Surprisingly the size of the particles causing the density fluctuation is less important (r5 = 13.8%) than the distance of the separation of these particles from each other (r7 = 34.7%). The size of the optical anisotropic units is also important (r8 = 33.8%) as well as the p7, the amplitude of the orientation fluctuation (r,, = 30.5%).

Similar statements can be made for the scattered intensity in the I, mode. It is most influenced by p3 (30%) and p5 (20%), the two amplitude measures for density and orientation fluctuation, respectively. As in the I,, mode, the I, is also less influenced by the size of the protein aggregates (r4 = 6.0%) than by their distance of separation (r5 = 23.4%).

The effect of the size of the optically anisotropic unit is also of the same order (r4 = 18.2%) as the effect of the volume fraction of the protein aggregates (r5 = 14.6%).

The dissymmetry of the light scattering is supposed to be influenced more by the size parameters than by the amplitude measures. We see that this is the case. When comparing the correlation coefficients of the I,, mode (1) to that of the dissymmetry (11), one sees that the correlation between the scattering and the amplitude measure decreases (r1,2 = 42.1% to r1,13 = 19.3%), while the correlation coefficients between scattering and the size parameters increases somewhat (r1,2 = 13.5 to r1,13 = 15.2). On the other hand, the correlations between the scattering and other size
parameters do not improve going from direct scattering intensity to dissymmetry.

With respect to the individual lenses, the correlation coefficients express the relationship between the scattering of individual sections and the particular optical parameter. As expressed in Part II, no overall generalization of nuclear cataracts can be made with assurance. Each individual lens shows its own particular correlation pattern. If anything, we can say that the dense nuclear cataracts in group I (lenses 98, 80, 73, 01) are mainly influenced by certain factors as well as by the separation of protein aggregates whereas in the group II cataracts (lenses 72, 67, 13, 12) in which opacity seems to form a ring around the center of the lens, no such group characteristics are observable. This again demonstrates the importance of the proper description and characterization of each cataractous lens.

Finally, one would like to identify the structural elements described by the optical parameters that can be related to structures seen in the electron microscope.

In the above discussion and in previous articles, we attributed the first correlation distance of the density fluctuation (a1) to the size of the protein aggregates. This we have done on the basis of calculated size of high molecular weight aggregates. That is, a molecular weight of $5 \times 10^8$ daltons would yield a 310 nm diameter. In cataractous lenses, one can see in the electron microscope aggregates of the order of 300 to 500 nm. The second correlation distance (a2) of the density fluctuation that varied between 400 and 1800 nm can be identified with the interparticle separation. The amplitude measure of the density fluctuation indicates the refractive index difference between the protein aggregate and its surroundings. When this measure is high, a syneretic process is operative in cataract formation. This means that due to aggregation (hydrophobic bond) and/or internal collapse (through crosslinking, entanglement of polypeptide chains), the degree of hydration of the protein aggregates decreases and the water leaving the aggregate decreases the refractive index of the surrounding and increases the refractive index of the aggregates. The volume fraction of the aggregate simply represents the average concentration of protein aggregates.

With respect to the size parameter of the optically anisotropic units that varied from 300 to 1800 nm, two interpretations can be advanced. Geometrically anisotropic bodies-microtubules, muscle-like proteins (actin), and intermediate filaments—have been reported in the literature. Such filaments and the muscle proteins proved to be optically anisotropic, and we may assume that in the lens they contribute to the structural birefringence. The question is whether all the optical anisotropy can be attributed to these filaments (model I) or do they act as birefringent bodies in conjunction with the protein (crystallin) aggregates (model II). We think that the second model is more plausible. Most of the orientation fluctuation correlation function of cataractous eyes could be fitted with a one-term exponential analytical expression. This means that the average size of the optically anisotropic units and their average separation are the same for most sections of the cataractous lenses. If only the microtubules, actins, and filaments would comprise the optically anisotropic bodies, one would expect a two-term exponential analytical expression for the orientation-correlation function similar to that we found in the density-correlation function. That is, one would expect that both the average size of the filaments and their average separation from each other would contribute and these average values would be different.

Therefore we can conclude that the cytoplasmic skeleton of birefringent filaments is entangled with the protein aggregates and possible orients them in an anisotropic manner and that together they constitute the optically anisotropic bodies. The fact that in the gross correlation coefficients the separation of the protein aggregates proved to be an important contributor toward the light scattering intensity while, on the whole, the volume fraction did not reinforce the above arguments. If the protein aggregates would be randomly distributed, then the average separ...
aration of these aggregates would be a measure of the concentration (volume fraction). The fact that the volume fraction and the average separation distance show different gross correlation coefficients indicates that the protein aggregates themselves may be anisotropic aggregates.

The $\delta^2$ or the amplitude measure of the orientation fluctuation then simply indicates the fluctuation in the preferential alignments of those optically anisotropic bodies.

In conclusion, the eight lenses with nuclear cataracts investigated have different contributions from the different optical parameters just as their clinical classification differ from each other. In general, we have seen two cases of nuclear cataracts: one group that has its maximum turbidity at the center of the lens and the other that has a ringlike turbidity around the center. The first group is more homogeneous and have more common feature than does the second.

In general, one can say that the aggregation process is not the most important contributor in nuclear cataractogenesis. The sizes of the protein aggregates are not much greater than those found in some normal lenses. The most important difference is that the protein aggregates are much more dense and the $\eta^2$ values are 10 to 100 times larger than in normal lenses. This implies that a syneretic process is the more significant contributor.

The second most important feature of cataractogenesis is the increase in the structural elements that contribute to the depolarized mode of scattering, $I_+$. The values of $\delta^2$ increased 100 to 6000-fold compared to the normal lenses. This occurs at the same time that the size of the optically anisotropic bodies is about the same or in many instances less than that found in normal lenses. This implies to us that although in normal lenses the cytoskeletal anisotropic filaments may be the chief contributor toward optical anisotropy, their small concentration and preferential alignment make small contribution toward scattering. On the other hand, in nuclear cataracts these filaments actinlike threads, and microtubules may be somewhat disaligned and entangled in the protein aggregates, and furthermore they may orient the aggregates into alignments that vary from point to point.

The third important contributor in nuclear cataract formation is the decrease in the separation of the protein aggregates compared to normal lenses. This implies that the concentration of the aggregates increases during cataractogenesis and that these aggregates are unevenly distributed in the lens, possibly aligned and entangled with the cytoskeletal filaments or the membrane components. Furthermore, such a directional aggregation may lead to direct association between membrane proteins and cytoskeletal filaments not unlike the one reported in other complex cellular phenomena.

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