Distribution of protein within lenses with x-ray cataract

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Cataracts were induced in rat lenses by irradiation with x-rays generated at 185 kv. A single exposure of 1,000 r or 500 r was directed toward one eye, the other being shielded and used as the control. Sections from control lenses and lenses in different stages of cataract were studied by quantitative microradiography. This method enables the distribution of protein to be determined down to a subcellular level within the lens. Compared to normal lenses, the concentration of protein was reduced in most parts of the cataractous lens, most prominently in mature cataracts. The salient features were sharp, steep gradients in the distribution of protein, usually situated in the peripheral cortex. A close correspondence was present between the position of these protein gradients and that of the opacities. In the microradiograms of cataractous lenses, morphological changes in the lens fibers were identified. The physical basis of the opacities in these cataractous lenses is discussed. It is concluded that the main loss of transparency is due to mechanisms of reflection in the steep protein gradients corresponding to interfaces between regions with different refractive indices.

Key words: radiation cataract, rats, radiation intensity, time factors, age factors, lens cortex, lens nucleus, lens fibers, lens capsule, proteins, histopathology, microradiography.

The crystalline lens is a very radiosensitive tissue, and cataracts may occur after moderate doses of ionizing radiation. The clinical and histological characteristics of radiation cataract in man and in experimental animals have been described by many authors. The morphological changes in the lens are strikingly similar in all species investigated. These radiation-induced injuries become clinically visible after a relatively long period. The length of this latency depends mainly on the dose of radiation but also, to some extent, on the age and species of the animal. The aim of the present investigation was to explore some of the associations between the altered optical properties and the structural and quantitative chemical changes in lenses with radiation cataract.

Optical properties of the lens, such as refractive index and transparency, are due mainly to the protein and water phases of the lens. Since these phases constitute more than 95 per cent by weight of the lens, their quantitative relations are of particular interest. The distribution of protein has been studied in the normal lens from rats of different ages by means of quantitative microradiography. This technique enables the concentration of protein to be determined in situ within thin lens sections down to a subcellular level. In the present study, rat lenses, representing various stages of radiation cataract, were investigated with this technique.
Material and methods

Twenty male Sprague-Dawley rats, 10 days of age, were exposed on the right eye to copper-filtered (0.5 mm Cu) x-rays generated at 185 kv from a tungsten anode. The dosage to the eye was 1,000 r in 10 animals and 500 r in the other 10. The dosage rate was 122 r per minute. The animals were sucklings and had closed eyelids at the time of irradiation. During the irradiation, the head of the animal was kept in a fixed position in a plastic tube. A lead shield with a circular hole, 8 mm. in diameter, in front of the right eye made it possible to protect the major part of the animal, particularly the left eye, from exposure to x-rays. The position of the animal was carefully controlled before, during, and after irradiation.

After the suckling period, the animals were fed on a standardized normal diet. The eyes were examined ophthalmoscopically or by slit lamp once a week as well as when the animals were killed. At different stages of cataract, the rats were killed by an overdose of ether. The removed lenses to be analyzed microdensitometrically were frozen immediately at about -140° C. in isopentane, cooled by liquid nitrogen. The frozen lenses were freeze-sectioned to a thickness of about 10 μ. The sections were usually taken parallel and close to the optic axis of the lens, but some lenses were sectioned perpendicularly to the optic axis. At least 3 sections from each lens were analyzed microradiographically. The sections were freeze-dried and mounted together with a reference system composed of a step wedge of thin mylar foils (490 μg/cm²). The thickness of each section was determined in at least 10 different areas. The microradiographical and densitometric procedures were identical to those utilized earlier.12-14 The elemental composition of 3 lenses with mature x-ray cataract was determined for carbon, hydrogen, and nitrogen (Mikroanalyslaboratoriet, Uppsala), the mean values being 52.2, 6.3, and 16.3 per cent by dry weight, respectively. These values differed only slightly from the mean values for the normal lens,12 and the same ratio—1.02—between the mass absorption coefficients for mylar and dry lens substance was obtained. From the densitometer readings the dry mass of the lens was calculated and expressed as mass per unit volume of protein.

The shrinkage in the freeze-drying procedure necessitated a correction factor of 10 per cent,12 to be used on the measurements for the protein content. No correction was made for the systematic error due to the difference in mass between protein and total dry material. The percentage of the main nonprotein constituent, lipids, does not differ greatly in the cataractous and the normal lens.15 Consequently, the error due to nonprotein constituents in the cataractous lens is of the same magnitude, ± 4 per cent, as in the normal lens. The contribution of the other systematic errors to the total error at microradiography can be estimated at about ±3 per cent at absolute determinations, and the total random error can be estimated at about ±5 per cent.12

Results

The development of x-ray cataract followed the pattern described in earlier investigations.1-7, 9-11, 16, 17 The clinical changes in the lenses examined by slit lamp, were divided into 4 stages of opacity according to Christenberry and Furth.26 In the lenses irradiated with a dose of 1,000 r of x-rays, the first signs of cataract—opacities at the posterior pole—became detectable after about 2 months. On transillumination, weak ringlike opacities in the middle of the cortex were disclosed one or 2 weeks later. The first mature cataract was observed after 9 months, and most lenses were totally opaque at 14 months after irradiation. In the early stages (1 and 2) of cataract, opacities were most frequently present fairly close to the capsule, particularly in the posterior cortex. Later, opacities were observed in the main part of the posterior and the anterior cortex (stage 3 and 4). The nucleus of the lens was, however, always transparent. The formation of opacities was relatively uniform in all the lenses, the most prominent lesion being a membranous opacity in the cortex some tenths of a millimeter inside the capsule. The rats irradiated with a dose of 500 r showed a much slower development of cataract, the first mature cataract being observed after 15 months. However, most cataracts were then still at stage 2.

The microradiographical appearance, shown in Figs. 1 and 2, and the quantitative determinations, given in Figs. 3 to 5 and Table I, are from animals irradiated with 1,000 r. For the corresponding stages of cataracts from animals exposed to half the dose, no essential differences in the results were detected.

Microradiographs of sections from cataractous lenses always showed differences compared with those from the correspond-
Protein distribution in x-ray cataract

In the first stage of cataract, these differences were local and not very prominent. Irregular and swollen lens fibers were observed close to the posterior capsule, and vacuoles were sometimes present at the anterior capsule (Fig. 1). In some of the sections, smooth lines or, actually interfaces, delimiting areas with slightly different absorption of x-rays were seen. They represented sudden gradients of x-ray absorption in the sections, separating a peripheral region of the cortex with a lower absorption from a region with higher absorption. The position of these interfaces within the lens had approximately the same position as the ringlike opacities, observed in the equatorial cortex on transillumination, and to the membranous opacities, seen on slit lamp examination.

In stages 2 and 3 of cataract, the microradiographical changes became more pronounced, particularly in the peripheral cortex (Fig. 1). The lens fibers were more irregular and swollen, and often had a low absorption of x-rays. Thick lens fibers with a relatively high x-ray absorption were, however, sometimes present in the inner part of the cortex. Steep and irregular absorption gradients were often observed in the peripheral part of the cortex, usually within 200 μ from the capsule.

The microradiographs from sections of mature cataracts showed all the morphological changes in the cortex noted in earlier stages, but to a still more advanced degree (Fig. 2). However, the nucleus of these lenses invariably had a normal microradiographical appearance. Outside the nucleus there was a zone with changed cellular architecture, containing many swollen lens fibers. Some of these had a higher x-ray absorption than the surrounding fibers, usually reaching the same magnitude as the corresponding regions in the normal lens. The main part of the cortex exhibited an irregular and disorganized structure. At a distance of 100 to 400 μ from the capsule, a very steep gradient of x-ray absorption was always observed. Peripheral to this wavy interface, the cortex generally had a very low x-ray absorption, but areas with a somewhat higher absorption were also observed.

Quantitative determinations of the dry mass in the lens were performed by densitometric evaluation of the microradiographs. Since protein constitutes about 96 per cent of the dry lens substance, the evaluated data were taken to represent the protein content. No corrections were introduced for the nonprotein components, as the optical properties are dependent on the total dry mass. Consequently, the values given for the protein content are about

Table I. Protein concentration (Gm.-cm.⁻³) in 5 zones from lenses at different ages and stages of cataract

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Stage of cataract</th>
<th>No. of lenses</th>
<th>Zone I</th>
<th>Zone II</th>
<th>Zone III</th>
<th>Zone IV</th>
<th>Zone V</th>
</tr>
</thead>
<tbody>
<tr>
<td>135</td>
<td>1</td>
<td>3</td>
<td>0.185 ± 0.022</td>
<td>0.565 ± 0.035</td>
<td>0.785 ± 0.007</td>
<td>0.591 ± 0.045</td>
<td>0.123 ± 0.040</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.279 ± 0.009)</td>
<td>(0.615 ± 0.011)</td>
<td>(0.869 ± 0.046)</td>
<td>(0.663 ± 0.037)</td>
<td>(0.319 ± 0.024)</td>
</tr>
<tr>
<td>160</td>
<td>2</td>
<td>2</td>
<td>0.288 ± 0.033</td>
<td>0.570 ± 0.009</td>
<td>0.714 ± 0.006</td>
<td>0.548 ± 0.037</td>
<td>0.170 ± 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.381 ± 0.016)</td>
<td>(0.574 ± 0.043)</td>
<td>(0.824 ± 0.014)</td>
<td>(0.572 ± 0.023)</td>
<td>(0.196 ± 0.011)</td>
</tr>
<tr>
<td>220</td>
<td>2-3</td>
<td>3</td>
<td>0.208 ± 0.010</td>
<td>0.614 ± 0.020</td>
<td>0.784 ± 0.025</td>
<td>0.603 ± 0.021</td>
<td>0.107 ± 0.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.288 ± 0.011)</td>
<td>(0.640 ± 0.037)</td>
<td>(0.532 ± 0.013)</td>
<td>(0.626 ± 0.039)</td>
<td>(0.263 ± 0.025)</td>
</tr>
<tr>
<td>250</td>
<td>4</td>
<td>2</td>
<td>0.165 ± 0.033</td>
<td>0.400 ± 0.073</td>
<td>0.761 ± 0.062</td>
<td>0.495 ± 0.063</td>
<td>0.235 ± 0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.295 ± 0.014)</td>
<td>(0.714 ± 0.081)</td>
<td>(0.577 ± 0.002)</td>
<td>(0.722 ± 0.005)</td>
<td>(0.303 ± 0.030)</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard error of the mean. The corresponding mean concentrations of protein in the control lenses are given within parentheses.

Zones I and V comprise the peripheral part, 100 μ thick, inside the capsule at the anterior and the posterior poles, respectively. Zones II and IV are situated halfway between the center of the lens and the anterior and posterior poles, respectively. Zone III comprises the center of the lens.
Fig. 1. For legend see opposite page.
4 per cent higher than those for the pure protein fraction.\textsuperscript{12}

The protein concentration was determined in circular areas 13.5 \( \mu \) in diameter and 100 \( \mu \) apart. These areas were situated along a straight line, starting close to the anterior pole of the lens, and ending close to the posterior pole. The amounts of protein were expressed in Gm.-cm.\textsuperscript{-3} and plotted in diagrams as a function of distance from the center of the lens. Some representative diagrams are given in Figs. 3 and 4 for different stages of cataract, as well as for the corresponding normal lens.

The development of x-ray cataracts was characterized by a reduced volume of the whole lens and a decrease in protein concentration in all parts of it. The more advanced was the cataract, the greater was the decrease in protein content (Figs. 3 and 4). In Table I, the mean value for the protein concentration in certain zones of the lens have been calculated for different stages of cataract and different ages. Within these zones the variation in protein content between individual lenses was relatively large (Table I), to be ascribed partly to the irregular local variations in protein concentration.

The x-ray absorption gradients represent interfaces between regions with different protein concentrations. These gradients were measured in the microradiographs with a scanning photometer (diameter of scanned area 3, 2 \( \mu \)) along lines at right angles to the interfaces. They were frequently found to be extremely sharp. The highest gradient measured was 0.32 Gm.-cm.\textsuperscript{-3} over a distance of less than 2 \( \mu \) in a lens with mature cataract. Protein gradients of the order of 0.20 Gm.-cm.\textsuperscript{-3} within a few microns were found in all lenses with cataracts in stages 3 and 4. A representative curve from a lens with mature cataract is given in Fig. 5. For cataracts in stages 1 and 2, these gradients were of lower magnitude, but were still distinct.

**Discussion**

Opacities in lenses with radiation cataract have been shown by many investigators to be concurrent with histological changes.\textsuperscript{1-11} Structural changes, such as irregular shape and size of the lens fibers, were here also identified in the whole cortex already at stages 1 and 2 (Figs. 1-2). When the first cataractous manifestations appeared, all the lens fibers, constituting the lens at the time of irradiation (rats 10 days old), were situated within the nucleus. The only microradiographically detectable change in the lens nucleus at any stage of cataract was the slightly reduced protein concentration. The decreased protein content in the central lens fibers may partly be an indirect effect of the damage to the cortex. Therefore, the direct radiation damage to the lens fibers, present at the time of irradiation, seems to cause only slight microradiographically detectable changes.

The most striking feature recognized in the microradiographs was the sharp cortical gradients in the concentration of protein. Minor gradients were noted already in the initial stages of cataract formation in the middle of the cortex. Major gradients appeared later in some regions close to the posterior capsule, as well as inside the anterior capsule. The mechanism causing these gradients after such a long latent period could not be revealed.

A loss of transparency must be due either to absorption or scattering of

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**Fig. 1.** A-F, Microradiograph from the peripheral part of the lens cortex, showing different cataractous changes. A and B show localized regions with swollen lens fibers in a lens with early opacification (stage 1). C and D show vacuoles close to the capsule and minor gradients of protein in cataractous lenses at stage 2. E and F show cataracts at stages 3 and 4 with distinct irregular interfaces between regions with different protein concentrations. The capsule is partly lost in some of the figures. The vertical dark lines are fractures in the freeze-dried sections. (Magnification \( \times320 \).)
Fig. 2. For legend see opposite page.
light. True absorption is highly unlikely in these cataractous lenses since it would imply a deposition of pigment substances. There is no evidence that such substances are deposited in amounts sufficient to cause selective absorption in the opacities. These microradiographical studies indicate that scattering of light, especially from large particles or interfaces, is the major cause of opacity. The steep gradients in protein concentration which were recognized in all cataractous lenses are related to changes in refractive indices. The refractive index can be calculated by utilizing the Gladstone-Dale formula, \( n = n_m + \alpha \cdot C \). In this case, \( n_m \) is the refractive index of water, 1.333; \( \alpha \) is the specific refractive increment for lens substances, taken as 0.0018; and \( C \) is the concentration of protein in grams per 100 ml. solution, here determined microradiographically. The sharpest protein gradient observed, 0.32 Gm.-cm.\(^{-3}\) over 2 \( \mu \), then corresponds to a difference in refractive index of 0.058. At the protein gradients common in the cataractous stages 3 and 4, about 0.20 Gm.-cm.\(^{-3}\) over a few micrometers, the difference in the refractive indices will then be of the order of 0.036. These sudden gradients in refractive index will cause a loss of light energy due to reflection, which can be considered as a special case of scattering from large particles.

The reflectance, \( \rho \), can be calculated for the idealized case of a sharp and smooth surface separating 2 media with different refractive indices. In the lens the interfaces are not perfect, and multiple reflection is often present. However, the reflectance would still be of the same magnitude as in the idealized case, for which it can be calculated from the following equation at normal incidence of light:

\[
\rho = \frac{I}{I_0} = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} \tag{1}
\]

Here, \( I \) and \( I_0 \) are the reflected and incident intensity of light, respectively, and \( n_1 \) is the refractive index of the first medium and \( n_2 \) that of the second medium. If \( n_1 \) is taken as 1.350 and \( n_2 \) as 1.386, corresponding to a protein gradient of 0.20 Gm.-cm.\(^{-3}\), the reflectance will be of the order of 0.02 per cent.

When light falls on the interface, separating regions with different refractive indices, the reflectance will increase with the angle of incidence according to the Fresnel-Maxwell equation:

\[
\rho = \frac{\sin^2(\phi - \phi')/\sin^2(\phi + \phi')}{\tan^2(\phi - \phi')/\tan^2(\phi + \phi')} \tag{2}
\]

Here, \( \phi \) and \( \phi' \) are the angles of incidence and refraction, respectively, measured relative to a perpendicular to the interface. If the angle of incidence increases, the reflectance will rise faster the closer the angle is to 90 degrees. Thus, for the same 2 refractive indices, the reflectance amounts to 11 per cent at an angle of 80 degrees and close to 100 per cent at 90 degrees. Consequently, the reflectance would become very low in all parts of the lens except in the equatorial region if the interfaces between the refractive indices are smooth and have the same shape as the outer surface of the lens. This applies even if the gradient of refractive index is high. However, the interfaces are usually wavy and irregular in the cataractous...
lenses (Figs. 3 and 4). Light will then fall on the interfaces under angles of incidence ranging from 0 to 90 degrees, and the reflectance will therefore vary considerably. On transillumination, the first sign of cataract was consistently a ringlike opacity in the equatorial region, which might be explained by the high angle of incidence of the light at the interface.

In addition to this kind of reflection, there will also be total reflection when light strikes an interface at an angle greater than the critical angle. The critical angle will be about 76 degrees if the refractive indices are $n_1 = 1.386$ and $n_2 = 1.350$ typical values at the interface in the posterior cortex of the cataractous lenses. Consequently, light forming an angle of 76 degrees or more with the perpendicular to the interface will be totally reflected with

![Graph](image_url)

Fig. 3. Distribution of protein along the optic axis in central sections from 3 lenses at different stages of cataract (filled circles) and from the corresponding control lenses (open circles).
this direction of light. Furthermore, the wavy interfaces will cause an irregular refraction which also results in a loss of transparency.

Scattering of light from small particles occurs if these have dimensions of the order of magnitude of the wavelengths of light or smaller. In the lens, the protein
molecules comprise the major fraction of small scattering particles. In the normal transparent lens, the proteins are assumed to have a high degree of spatial order, which would explain why only about 10 per cent of the light energy is scattered, as compared with 70 per cent if independent scattering for each molecule is assumed. This high spatial order of the proteins is probably destroyed in major portions of the cortex from lenses with x-ray cataract at later stages, since the cellular architecture and the distribution of proteins are more irregular. Scattering of light from disorganized small particles would, however, be homogeneously distributed over the whole cortex, and not be localized to discrete opacities. Although the magnitude of small particle scattering is unknown, it can be presumed that it does not constitute the major part of the additional loss of light energy in the discrete opacities.

It is, therefore, good evidence for the main part of the loss of transparency in radiation cataracts being ascribed to mechanisms of reflection in the interfaces between regions with different protein concentrations. In order to confirm this conclusion, further experiments are in progress to measure the intensity of light scattered from different parts of cataractous lenses.

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REFERENCES