The effects of ionizing irradiation on lens cation permeability, transport, and hydration

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The effect of in vivo x-irradiation on rabbit lens permeability was studied by the \( \text{\^{86}Rb} \) runout method. At 24 hours following x-irradiation of the lens the rate of \( \text{\^{86}Rb} \) runout was found to be increased and reached a maximum at one week. Over the subsequent 2 weeks the rate of \( \text{\^{86}Rb} \) runout decreased, although still remaining above normal. The increase in lens permeability preceded the appearance of lens opacities and was found to be dose dependent. The active transport of cations as measured by \( \text{\^{86}Rb} \) uptake was unaffected. Alteration in lens permeability was accompanied by an increase in lens water. The effect of in vitro B irradiation on the permeability of the anterior or posterior lens surface was investigated and found to parallel in vivo x-irradiation to the entire lens.

Posterior subcapsular lens opacities have been described clinically as the result of excessive exposure to ionizing irradiation. Many investigators have been successful in producing radiation cataracts in laboratory animals, most notably the rabbit. As yet, the mechanism for such an alteration in the normal lens exposed to ionizing irradiation is not completely understood.

Sheppard and Beyl demonstrated that x-irradiation of red blood cells caused an increase in membrane permeability to univalent cations, without affecting the normal cation active transport. Other investigators have demonstrated increased cellular permeability to cations with exposure to ionizing irradiation in skeletal muscle, heart muscle, and yeast.

Alteration in lens permeability producing increased lens hydration is thought to initiate the development of other forms of lens opacities. The purpose of this present study is to evaluate in the rabbit lens the changes in cation permeability, the active transport of cations, and hydration following exposure to ionizing irradiation.

Methods and materials

The right eyes of 1 1/2 pound albino rabbits received 1,000, 3,000, or 6,000 r x-irradiation according to the following radiation factors: Van Guard 280 kv., 19 ma.; HVL 1.53 mm. Cu; 22 cm. target to sample distance at 900 r per minute. The left eyes were outside the x-ray beam and received approximately 1 per cent of the dose delivered to the right eye as measured by a lithium fluoride microrod dosimeter placed in the left posterior chamber in a typical experimental situation.

\( \text{\^{86}Rb} \) runout following x-irradiation. The rubidium runout experiments were patterned after the procedures described by Becker and Cotlier.
intervals of 2 hours, 7, 14, and 21 days following x-irradiation, the rabbits were killed and both the irradiated and control lenses were excised. Each lens was transferred to a Merriam-Kinsey tube containing 10 ml of medium. TC-199 with bicarbonate (obtained from Microbiologic Associates) made up 68 per cent of the incubation medium and the rest was made up so that the final concentration of bicarbonate ion was 29 mM, calcium ion 1.25 mM, and glucose was 5.5 mM. The medium was equilibrated with 95 per cent air and 5 per cent CO₂. Sufficient isotopic rubidium (⁶⁷⁸Rb) (obtained from Isoserve Corp., Cambridge, Mass.) was added to the medium to give approximately 100,000 c.p.m. per milliliter of medium. The lens pairs were incubated at 37.5° C. for 24 hours whereupon the radioactive medium was replaced with an identical medium without ⁶⁷⁸Rb but containing 10⁻⁴M ouabain. Aliquots of the new medium were assayed for radioactivity at various times up to 4 hours when the lenses were removed from the culture tubes, weighed, homogenized in 3 ml. trichloroacetic acid, and centrifuged. Aliquots of lens supernatant along with the samples of the medium were counted in a liquid scintillation counter. After correcting for background, the results were expressed in counts per minute per 10 ml of the medium, and the lens results were expressed in counts per minute per lens.

The per cent runout of total lens ⁶⁷⁸Rb recovered in the medium, a measure of cation permeability, was calculated as follows: total counts per minute recovered in medium divided by total counts per minute initially present in the lens x 100 = per cent runout of total ⁶⁷⁸Rb recovered in the medium at the fourth hour. The total initial lens counts per minute was equal to the sum of the counts per minute in the lens and the total counts per minute recovered in the medium after 4 hours of incubation. The runout values were calculated for irradiated and control lenses at the hourly intervals, and the standard error of their means was calculated by the formula:

$$S.E. = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

**Rb accumulation following x-irradiation.** At intervals of 2 hours and 3, 4, 7, and 28 days following x-irradiation the rabbits were killed and the lenses removed. The lenses were transferred to the Merriam-Kinsey tubes each containing 10 ml of medium identical to that already described except that counts per minute of ⁶⁷⁸Rb was approximately 30,000 per milliliter of medium. The lens pairs were incubated at 37.5° C. for 4 hours and treated in the manner described above. The radioactivity of the medium and the lens was determined as described above. The results were expressed as the concentration ratio of the lens to medium as follows:

$$L/M = \frac{\text{c.p.m./ml. lens H}_2\text{O}}{\text{c.p.m./ml. medium}}$$

**⁶⁷⁸Rb runout following B-irradiation.** Albino rabbits (of approximately 1½ pounds each) without previous exposure to radiation were killed and the lenses removed. From each rabbit one lens
served as a control and the other was experimental; this constituted a lens pair. The experimental lens was transferred to a B-irradiation apparatus as depicted in Fig. 1. In this apparatus it was possible to deliver 16,000 r to either the anterior or posterior lens surface while the opposite surface received only 270 r. In this way it was possible to study not only the direct effect of ionizing B-irradiation on the lens, but also to study the effect of ionizing radiation on a single lens surface. The experimental lens after receiving 16,000 r B-irradiation and the control lens were transferred to long term incubation tubes as illustrated in Fig. 2. These tubes contained 15 c.c. of the medium described previously and the contents of the tube were maintained at 37.5° C. for the duration of the experiment—7 days. The medium was changed every other day by transferring the lenses and platform-stopper units to sterile tubes containing sterile medium. At 6 days following irradiation the lenses were incubated for 24 hours in a medium containing 100,000 c.p.m. per milliliter ⁶⁷⁸Rb following which they were transferred to a Merriam-Kinsey tube with non-radioactive medium where a ⁶⁷⁸Rb runout was performed as described above.

In cases where the ⁶⁷⁸Rb runout was measured within the first 24 hours following B-irradiation, the lenses were transferred immediately to the Merriam-Kinsey tube after B application.
Table I. The effect of x-irradiation on $^{86}$Rb uptake

<table>
<thead>
<tr>
<th>Dose</th>
<th>Time duration following radiation (days)</th>
<th>Lens pairs</th>
<th>Control $\text{L/M}^*$</th>
<th>Radiation $\text{L/M}$</th>
<th>Radiation $\text{L/M} \times 100$ Control $\text{L/M}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,000r</td>
<td>2 hours</td>
<td>8</td>
<td>4.32</td>
<td>4.77</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>4.5</td>
<td>4.7</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8</td>
<td>4.27</td>
<td>4.46</td>
<td>104</td>
</tr>
<tr>
<td>3,000r</td>
<td>2 hours</td>
<td>7</td>
<td>4.77</td>
<td>4.72</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>5.38</td>
<td>4.93</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6</td>
<td>3.58</td>
<td>3.65</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>3</td>
<td>3.33</td>
<td>3.08</td>
<td>92</td>
</tr>
</tbody>
</table>

$L/M = \frac{\text{counts per minute per cubic centimeter lens H}_2\text{O at 4 hours of lens exposure to } ^{86}\text{Rb}}{\text{counts per minute per cubic centimeter medium}}$

Fig. 6. $^{86}$Rb runout 7 days following in vitro 16,000r x-irradiation to the posterior lens surface. Five lens pairs are plotted with each mean value bracketed by its standard error.

Results

$^{86}$Rb runout following x-irradiation. The results of a $^{86}$Rb runout experiment shown in Fig. 3 demonstrate a marked increase in the $^{86}$Rb permeability produced by exposure to 6,000r 7 days following x-irradiation exposure. Those lenses receiving 6,000r show approximately a 40 per cent increase in permeability compared to the controls. Fig. 4 illustrates a 30 per cent increase in runout in lenses receiving 3,000r x-irradiation 7 days following exposure. However, at 1,000r no detectable change in permeability is observed 7 days after exposure.

The increase in permeability could be detected within the first 24 hours. That the degree of change in cation permeability is dependent upon the time after exposure and on the dose is shown in Fig. 5. It shows the altered permeability state of the X-ray exposed lenses to be maximum.
at the end of the first week and then declining to lower levels at 2 and 3 weeks. The dose effect is shown by the fact that the runout at 6,000r is consistently greater than 3,000r and both are greater than 1,000r which shows no change.

All lenses following 6,000 and 3,000r x-irradiation are without lens opacities to direct ophthalmoscopy and slit lamp examination until 18 days following exposure when 50 per cent of the 6,000r and 3,000r have opacities. By 21 days all lenses exposed to 6,000 and 3,000r have posterior subcapsular opacities.

\(^{86}\text{Rb} \text{uptake following x-irradiation.}\) The effect of x-irradiation on the transport of cations by the lens is shown in Table I. Following doses of 6,000 and 3,000r at intervals of 2 hours to 28 days the uptake of \(^{86}\text{Rb}\) is not significantly affected. This is appreciated by the close similarity between the L/M ratio of the control and the irradiated lenses. The L/M ratio is an expression of the lenses ability to concentrate \(^{86}\text{Rb}\) against a concentration gradient. This L/M ratio is computed following a 4 hour exposure of the lens to \(^{86}\text{Rb}\). After 4 hours the increased permeability of the irradiated lens would decrease the ability of the lens to concentrate \(^{86}\text{Rb}\). The relative \(^{86}\text{Rb}\) uptake efficiency of the irradiated lens to the control is given in the last column of Table I. The results indicate the lack of a significant alteration inactive transport in those lenses exposed to ionizing irradiation. Even in those lenses which are cataractous (3,000r at 28 days), the uptake of \(^{86}\text{Rb}\) remains unchanged.

\(\text{B-irradiation to the anterior or posterior surface.}\) The effect of B-irradiation to the posterior lens surface on the cation permeability is shown in Fig. 6. Irradiated posterior lens surfaces leak \(^{86}\text{Rb}\) at a greater rate than do the control lenses. At 7 days following in vitro exposure the lens with the irradiated posterior surface shows a 25 per cent increase in \(^{86}\text{Rb}\) runout over the control. While the ionizing dose of 16,000r is high, it can be shown from Fig.: that only a small portion (the apex of the lens curve) receives this high dose, and the level of irradiation to the remainder of the lens surface drops off markedly as the equator is approached.

Similar to the in vivo x-irradiated whole lenses, the in vitro B-irradiated lens surfaces demonstrate a greater runout at one week than at one day following irradiation. This is illustrated in Fig. 7 which also demonstrates the slightly greater leak at the posterior as compared to the anterior surface for an equal amount of irradiation.

\(\text{Lens hydration.}\) An increase in lens H\(_2\)O might be expected with the alteration in lens permeability as demonstrated in the \(^{86}\text{Rb}\) runout studies. Lenses at one hour and at 28 days following 6,000r are analyzed with respect to per cent of lens water and dry weight and compared in Table II. There is a small but significant
gain in lens water and a decrease in lens dry weight. That this water gain, although small, is real is best appreciated by examining a histologic section of the posterior surface of a lens with a posterior subcapsular opacity which had received 6,000r in vivo 18 days previously (Fig. 8). It reveals that only the surface fibers of the posterior cortex are overhydrated. Electronmicrographs, Figs. 9A and 9B, of the posterior surface seen in histologic sectioning (Fig. 8) show intracellular accumulation of fluid with rupture of the fiber membranes.

The decrease in dry weight seen in Table II at 28 days indicates that irradiation appears to affect the normal rate of protein synthesis.

Discussion

The results of the ⁸⁶Rb runout experiments suggest that one of the early changes in the development of radiation cataracts is an alteration in lens membrane permeability. The mechanism whereby ionizing radiation affects lens membrane permeability is as yet unclear, but that permeability change may be a significant factor in the pathogenesis of radiation cataracts is

Fig. 8. A, Posterior rabbit lens surface 18 days following 6,000r in vivo x-irradiation; note vacuolization of surface fibers. B, Normal posterior lens surface (sections, staining, and photographs by Dr. T. Kuwabara).
suggested by the following observations. The permeability of the lens undergoes a significant increase in the first 24 hours. The increased permeability leads to a gain in lens water. Although small in terms of the total lens, this water gain is significant and appears confined to that portion of the lens at the surface and exposed to the external milieu. Electronmicrographs of these fibers at the posterior surface at the

Fig. 9A. Posterior lens fibers 18 days following in vivo x-irradiation. Note intracellular fluid collection (if) with disruption of lens fiber membranes (rm). Posterior capsule (pc) (sections, staining, and photographs by Dr. T. Kuwabara). (Original magnification ×20,000.)
stage of nascent opacification show not only an increased fiber hydration, but rupture of membranes. These photographs suggest that the lens fibers may have ruptured secondary to increased hydration as the result of increased permeability.

The finding of altered lens permeability immediately following irradiation and before the actual existence of lens opacities contrasts with the observations of von Sallmann and Loeke, who were unable to detect a significant alteration in lens per-
meability until the process of lens opacification had become manifest. The $^{85}\text{Rb}$ method of detecting cation changes used in our experiments is much more sensitive than the procedures available to von Sallmann and the fact that he was working at slightly lower doses may account for the discrepancy in results.

Cogan and co-workers have suggested that the posterior lens opacity is the result of migration of aberrant swollen epithelial cells to the posterior pole. However, Hanna has shown with tritium-labeled epithelial cells at the time of irradiation exposure that the opacity was already present before epithelial cells had migrated to the area of the opacity. We also do not find epithelial cell migration in the area of the posterior pole at the initial onset of lens opacification, but do find the disrupted lens fibers at this time. The swollen epithelial cells, presumably also the result of altered permeability, may migrate to the area of fiber disruption to occupy the space vacated by a viable fiber.

An interesting feature of the $^{85}\text{Rb}$ run-out studies is that a maximum increase in permeability occurs one week following irradiation. The posterior surface fibers appear in the histologic sections to be more susceptible to swelling secondary to receiving ionizing radiation. A possible explanation for this phenomenon is that they are in direct contact with the external environment. Such a position submits them to chronic exposure to ionic concentrations different from their own, whereas the deeper fibers may not be similarly exposed. As their ionic concentration increases and their water content increases there may be a further disruption of the membrane, hence, an increase in permeability reaching its peak at one week.

The recovery in membrane permeability after the first week may be explained by the continued growth of the lens following irradiation. In Fig. 10 new fibers having developed from epithelial cells appear to cover the damaged surface fibers at the posterior pole. It is seen in Fig. 10 that there are normal-appearing lens fibers overlaying the damaged fibers. These new fibers appear to cover the "leaking" surface fibers thus interposing an intact membrane between the leaking lens fibers and the environment.

The lens subjected to ionizing radiation was shown to have normal $^{85}\text{Rb}$ active transport even into the stage of lens opacification. This indicates the lack of importance of deranged active transport in the over-all pathogenesis of radiation cataracts.

Several points of interest arise from the results obtained with B-irradiation of a single lens surface in vitro. The alteration in permeability is similar to that of the x-irradiated whole lens in vivo which indicates that ionizing effects of both B and x-irradiation produce the same type of effects on rabbit lens membranes. It also answers the question as to whether the changes in the lens leading to opacification are indirect, secondary to changes in aqueous humor—its composition or dynamics—and/or vitreous, or whether they are direct. Irradiation in these in vitro experiments was carried out in an artificial medium which was changed when the lenses were transferred to their incubation tubes. These experiments indicate that radiation can

Table II. The effect of x-irradiation on lens H$_2$O and lens dry weight

<table>
<thead>
<tr>
<th>Dose</th>
<th>Time following radiation</th>
<th>Mean wet lens weight</th>
<th>Mean dry weight</th>
<th>Mean lens H$_2$O</th>
<th>% Lens H$_2$O</th>
<th>% Lens dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 hour</td>
<td>196.69</td>
<td>61.7</td>
<td>134.99</td>
<td>68.7 ± 0.3</td>
<td>31.3 ± 0.3</td>
</tr>
<tr>
<td>6,000r</td>
<td>1 hour</td>
<td>196.45</td>
<td>61.96</td>
<td>134.49</td>
<td>68.7 ± 0.3</td>
<td>31.3 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>28 days</td>
<td>272.68</td>
<td>90.85</td>
<td>182.35</td>
<td>66.8 ± 0.42</td>
<td>33.2 ± 0.42</td>
</tr>
<tr>
<td>6,000r</td>
<td>28 days</td>
<td>205.02</td>
<td>86.21</td>
<td>178.74</td>
<td>67.7 ± 0.26</td>
<td>32.3 ± 0.26</td>
</tr>
</tbody>
</table>
Fig. 10. Posterior lens fibers 18 days following 6,000 r in vivo x-irradiation. Note intact fiber membranes (nsf) just beneath the posterior capsule (pc) with underlying intracellular fluid (if) collection and disruption of lens fiber membranes (rm) (sections, staining, and photographs by Dr. T. Kuwabara). (Original magnification x14,400.)
affect the lens directly but do not rule out the possibility that indirect effects can also influence the course of cataract development.

Both the anterior and posterior irradiated lens surfaces are seen to have a similar leakout pattern, although the posterior surface appears to be affected to a slightly greater extent. This indicates that permeability characteristics of the two lens surfaces as affected by ionizing irradiation are similar. This leads to the question: If the anterior and posterior surfaces are similarly affected by ionizing irradiations, why are the lens opacities more common in the posterior subcapsular area? The experimental results obtained suggest the following possible explanation. Kinsey and Reddy\(^{10}\) have shown that by far the majority of active transport occurs across the anterior surface at the site of the lens epithelium. If both the anterior and posterior surfaces undergo an alteration in permeability to cations as these experiments indicate, since the anterior surface alone possesses the capacity to correct the large ionic shift, then the increased hydration secondary to increased intracellular tonicity will occur only posteriorly in the surface cortical fiber layer where the lens opacities are seen to occur. The slightly greater alteration in permeability posteriorly may also cause increased hydration of the posterior fibers.

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**REFERENCES**