The effect of ionizing radiation on protein and carbohydrate metabolism in the rat lens

Sidney Lerman, Anima Devi, and Renaka Banerjee

The effects of ionizing radiation on the rat lens were investigated by means of single 1,500 r whole body or head exposures. The results indicate that there is no marked alteration in carbohydrate metabolism within the first week or two after such exposures to x-ray. A marked increase in the incorporation of C-14-leucine only into the insoluble protein-RNA complex of such lenses has been shown to occur within 6 hours after irradiation. A similar increase in the incorporation of P-32 into this albuminoid RNA* also becomes manifest 6 hours after exposure to x-ray.

Radiation cataractogenesis has been subject to extensive histopathologic and histochemical studies during the last two decades.1-16 These investigations have shown that the initial changes in such lenses occur mainly in the epithelial layer, and cataracts develop because of pathologic alterations in these cells. Probably the most striking changes occur within the cell nuclei, particularly in those cells located at the equatorial zone.5,9-13 Von Sallman17-19 has demonstrated that microscopically apparent early radiation damage precedes the development of clinically visible lenticular opacities by many days or weeks. He noted the rapid and marked inhibition of cellular mitosis which develops shortly after exposure to x-ray and the occurrence of nuclear fragmentation, degeneration, and extrusion of chromatin material from these cells which becomes manifest shortly thereafter. Von Sallman suggested that the pathogenesis of x-ray cataracts might be explained by postulating two sets of lesions. The primary lesion would be located in the cell nuclei of the germinative zone, whereas the secondary stage, which leads to destruction of the lens fibers and eventual opacification, could be due to chemical changes. He proposed that these latter changes might be based on an alteration in the permeability of certain phase boundaries within the lens.20

On the basis of extensive biochemical investigations, Pirie21 has also concluded that the development of radiation cataracts may proceed in two stages; the first is due to the direct effects on the epithelium, while the second stage involves damage to the lens fibers. She suggested that many of the biochemical alterations observed during the course of the development of x-ray cataracts may belong to the second stage.

The most striking initial biochemical change observed in the lens after exposure to x-ray has been the rapid fall in SH groups.21-23 The most marked decline occurs in the concentration of glutathione. How-

*Dische and associates recently reported in the Am. J. Ophth. 51: (Pt. 2)993, 1961, on the occurrence of albuminoid RNA in the rat lens. This prompted our study on albuminoid RNA in this paper.
ever, the activity of most of the glutathione-requiring enzymes does not decrease at a similar rate. Pirie\textsuperscript{21} assayed many of the enzyme systems required for anaerobic glycolysis and the Krebs cycle and was unable to find a significant decline in their activities until lenticular opacities became grossly manifest. She was also unable to detect any early changes in the level of high energy phosphates in such lenses,\textsuperscript{27} nor could she find any alterations in the concentration of many of the necessary coenzymes.\textsuperscript{21} She corroborated the finding of Dische and others\textsuperscript{28} that there is a slow, progressive decline in the level of soluble protein in x-irradiated lenses.

In an attempt to elucidate some of the biochemical mechanisms which may be responsible for the pathogenesis of radiation cataract, certain areas in protein, ribonucleic acid (RNA), and carbohydrate metabolism were investigated in the lenses of rats which had been exposed to a single cataractogenic dose of x-radiation.

Materials and methods

A. C-14-leucine incorporation experiments. Holtzman strain white male rats 5 to 6 weeks old which weighed approximately 60 to 65 grams each were employed in all the experiments. A single exposure of 1,500 r was used; some of the rats received whole body radiation; others had only their heads subjected to this dose of irradiation. In the latter group, pentobarbital sodium was employed for anesthesia, and the controls for these experiments also received a similar dose of this barbiturate. Except for water, all the rats were not fed for the first 24 hours after exposure and the controls were treated in a similar fashion. The animals were injected with 0.2 ml. C-14-leucine (specific activity $2.5 \times 10^6$ c.p.m. per milliliter) intraperitoneally at specific time intervals after irradiation as indicated in the results, and were sacrificed 3 hours after the injection. Their lenses were removed immediately, carefully blotted, weighed, and homogenized in Dounce microhomogenizers. The methods employed in determining the degree of recovery of the labeled soluble and insoluble RNA-protein fractions were described in another communication.\textsuperscript{29} Both lenses from one animal were used for each experiment.

B. P-32 incorporation experiments (RNA metabolism). The incorporation of P-32 into the various RNA fractions (soluble RNA, microsomal RNA, and albuminoid RNA) of the rat lens was studied in a second group of animals. These animals received 1,500 r of whole body or head irradiation as described above. Each animal received 50 $\mu$C P-32 by intraperitoneal injection immediately after irradiation and was sacrificed either 6 or 24 hours later. Control rats were treated in a similar manner. The lenses were immediately removed, carefully blotted, weighed, and homogenized in Dounce microhomogenizers containing 3 to 4 ml. of 0.25M sucrose plus a trace of magnesium. The albuminoid fraction was removed by centrifugation at 600 G for 15 minutes in a refrigerated centrifuge and washed twice with 4 ml. of the sucrose solution. The albuminoid RNA was extracted from the final precipitate by Schneider's method.\textsuperscript{30} The supernatant fluids were pooled and centrifuged at 105,000 G for 2 hours in a Spinco centrifuge. The supernatant fluid was retained for extraction of the soluble RNA by the Schneider procedure, and the precipitate was similarly treated to extract the microsomal RNA. Six lenses from 3 rats were employed for each experiment. One milliliter aliquots from each of the 3 RNA fractions were retained for spectral analysis and the orcinol reaction.\textsuperscript{31} The methods employed in preparing the samples for counting have been described in another communication.\textsuperscript{29}

C. Carbohydrate metabolism. Single lenses from irradiated and control rats were incubated with C-14-glucose labeled in the number 1 or 6 carbon atom and the degree of recovery of C-14-O\textsubscript{2} after an incubation period of 21 hours was determined. The material and methods employed in this procedure have been reported in a previous communication.\textsuperscript{32} Lactate determinations were performed on a second group of lenses derived from animals exposed to 1,500 r head irradiation at weekly intervals. Sippel's \textsuperscript{33} modification of the Barker and Summerson \textsuperscript{34} method for lactate assays was used. The activity of the enzyme glucose-6-phosphate dehydrogenase was determined according to the method of Clock and McLean.\textsuperscript{35}

Results

A. The results of the experiments with C-14-leucine incorporation into the soluble and insoluble protein–RNA complex of the rat lens are shown in Table I. The degree of incorporation of this amino acid into the soluble fraction 6 hours after head irradiation was similar to that in the control lens. In the lenses derived from rats subjected to whole body radiation, there was a considerable decline in the uptake of C-14-leucine into the soluble protein–RNA complex. However, there was a marked
increase in the 6 hour incorporation of C-14-leucine into the insoluble protein–RNA complex of lenses derived from both groups of irradiated rats as compared with the control levels.

The 24 hour postirradiation incorporation experiments showed a fall in the amount of C-14-leucine incorporated into the insoluble fraction, while the soluble fraction showed no statistical difference between the control and irradiated lenses.

B. The results of the uptake of P-32 into various RNA fractions of the rat lens are shown in Table II. Since the soluble and microsomal fractions showed no change in the amount of P-32 recovered from the control or the irradiated lenses, only the data for albuminoid RNA have been tabulated. The concentrations of the three RNA fractions are also shown in Table II. Although the relative concentrations of all three remained unchanged in both the irradiated and control lenses, there was a marked increase in the degree of incorporation of P-32 into the albuminoid RNA from the irradiated lenses as compared with the control lenses. This increase was maintained for at least 24 hours after irradiation.

C. The results of the recovery of C-14-O₂ from control and experimental lenses incubated with glucose-1-C-14 or glucose-6-C-14 are presented in Table III as the C₃C₆ ratios. The activity of the enzyme G-6-P dehydrogenase and the lactate concentrations of two similar groups of lenses are also included in this table. The results of all these experiments indicate that there is no change in two of the three parameters studied in the normal and irradiated lenses. There is an increase in lactate concentra-

Table I. The incorporation of C-14-leucine into the soluble and insoluble protein-RNA fractions of the rat lens

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Average lens weight (mg.)</th>
<th>Type of exposure</th>
<th>Time sacrificed* (hr.)</th>
<th>Soluble protein-RNA complex in c.p.m./Gm. wet weight of lens</th>
<th>Insoluble protein-RNA complex in c.p.m./Gm. wet weight of lens</th>
<th>Ratio soluble/insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>19.5</td>
<td>Control</td>
<td>6</td>
<td>1,085 [900-1,050]</td>
<td>135 [50-250]</td>
<td>8.0</td>
</tr>
<tr>
<td>6</td>
<td>19.7</td>
<td>Head irradiation</td>
<td>6</td>
<td>1,115 [650-1,880]</td>
<td>515 [250-900]</td>
<td>2.2</td>
</tr>
<tr>
<td>3</td>
<td>Whole body irradiation</td>
<td>6</td>
<td>715 [580-865]</td>
<td>210 [125-250]</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>19.2</td>
<td>Control</td>
<td>24</td>
<td>915 [780-1,160]</td>
<td>150 [140-170]</td>
<td>6.0</td>
</tr>
<tr>
<td>6</td>
<td>18.8</td>
<td>Head irradiation</td>
<td>24</td>
<td>1,038 [500-1,450]</td>
<td>107 [70-160]</td>
<td>9.7</td>
</tr>
<tr>
<td>5</td>
<td>Whole body irradiation</td>
<td>24</td>
<td>907 [635-1,055]</td>
<td>95 [80-110]</td>
<td>9.5</td>
<td></td>
</tr>
</tbody>
</table>

*All the animals were injected with 0.2 ml of C-14-leucine 3 hours prior to sacrifice.

Table II. The incorporation of P-32 into soluble, microsomal, and albuminoid RNA in the rat lens

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Time sacrificed after injection (hr.)</th>
<th>Albuminoid RNA (c.p.m./mg. total RNA)</th>
<th>Concentration* of RNA</th>
<th>Average lens weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Soluble RNA</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>1,132</td>
<td>1,040-1,200</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1,034</td>
<td>848-1,360</td>
<td></td>
</tr>
<tr>
<td>Head irradiation</td>
<td>6</td>
<td>1,060</td>
<td>3,480</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1,768</td>
<td>2,160-2,400</td>
<td></td>
</tr>
</tbody>
</table>

*The concentration of RNA is expressed in micrograms per lens.
Table III. The C\textsubscript{i}/C\textsubscript{0} ratio of C-14-O\textsubscript{2} recovered from normal and irradiated rat lenses incubated with glucose-1-C-14 and glucose-6-C-14 for 21 hours. The activity of G-6-P dehydrogenase and the lactate content of 2 similar groups of lenses

<table>
<thead>
<tr>
<th>Time sacrificed after exposure</th>
<th>C\textsubscript{i}/C\textsubscript{0} ratio</th>
<th>Lactate µg/lens</th>
<th>G-6-P dehydrogenase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Control (head exposure)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hours</td>
<td>8.91</td>
<td>8.52-9.31</td>
<td>30.86</td>
</tr>
<tr>
<td>24 hours</td>
<td>6.38</td>
<td>6.35-6.40</td>
<td>46.40</td>
</tr>
<tr>
<td>7 days</td>
<td>7.86</td>
<td>6.60-9.00</td>
<td>26.60</td>
</tr>
<tr>
<td>15 days</td>
<td>9.65</td>
<td>8.80-10.5</td>
<td>46.40</td>
</tr>
</tbody>
</table>

*The enzyme activity is expressed as the change in optical density at 340 µm of 0.001 per minute at 24°C per lens.

Discussion

The results of the experiments relating to carbohydrate metabolism in the lens during the first week or two after irradiation are in general agreement with the findings reported by Pirie.\textsuperscript{21} She studied many of the enzyme systems involved in anaerobic glycolysis and the Krebs cycle and found no change in their activities until lenticular opacities became clinically manifest. The increased lactate content in those lenses which had been irradiated 1 week earlier suggests that there might be an increase in the rate of anaerobic glycolysis at this time. This phenomenon may be associated with a more generalized attempt at recovery and regeneration which has been observed in many of the histologic studies on radiation cataract cited previously. The unchanged recovery of C-14-O\textsubscript{2} from glucose-1-C-14 and the normal activity of G-6-P dehydrogenase throughout the period under study indicate that the direct oxidative pathway does not participate in the recovery phase, nor is it directly affected by x-irradiation within the first 2 weeks. Anaerobic glycolysis is not only unaffected by radiation, but may be capable of an increased activity after exposure to radiation.

Aside from a significant fall in glutathione one which occurs 24 hours after exposure to radiation,\textsuperscript{21-24} attempts at determining other early biochemical alterations have heretofore been rather disappointing. The results of the amino acid incorporation experiments and the studies on RNA metabolism (as reflected by the degree of P-32 uptake into the three lenticular fractions) indicate that ionizing radiation also affects specific sites of RNA and protein metabolism in the rat lenses very soon after their exposure. Six hours after these animals have been radiated, there is a marked increase in the incorporation of C-14-leucine into the insoluble protein-RNA fraction of the lens which apparently disappears by 24 hours. Aside from the decrease in activity of the soluble protein-RNA complex which occurs 6 hours after whole body exposure, this fraction remains essentially unchanged. Since C-14-leucine was given intraperitoneally, it is possible that there might be an initial alteration in its rate of absorption after whole body radiation and this may explain the decrease in uptake noted in the 6 hour soluble fraction. In spite of such a decrease, the recovery of C-14-leucine in the corresponding 6 hour insoluble fraction is still higher than the control value. If these data are tabulated as the ratio of activity recovered in the soluble versus the insoluble components (Table I), the marked change which occurs in both of the 6 hour insoluble fractions is perhaps more readily apparent.
This ratio which varies from 6.0 to 9.7 in all the other experiments (both control and irradiated) shows a marked decline 6 hours after the animals have been exposed to both forms of radiation (whole body or head exposure). The decline to 2.2 and 3.4 in the head and whole body exposures, respectively, is mainly due to an increased incorporation of C-14-leucine into the albuminoid-RNA complex of these lenses.

In studying the metabolism of soluble, microsomal, and albuminoid RNA in such lenses, a somewhat similar situation is apparent. Although the concentration of each fraction remains unchanged (as measured by the orcinol reaction), there is a marked increase in the uptake of P-32 into albuminoid RNA after both types of radiation exposures. This increase, which occurs only in the albuminoid RNA again becomes manifest 6 hours after radiation. In contrast to the C-14-leucine experiments, this increase is maintained for at least the first 24 hours.

The results of the foregoing experiments indicate that ionizing radiation exerts an initial marked effect on lenticular albuminoid RNA which becomes evident as early as 6 hours after exposure. On the basis of the P-32 experiments, it would appear that there is a marked increase in the turnover (or metabolism) of the albuminoid RNA as reflected by an increased incorporation of P-32 into this molecule, while the specific concentration of this RNA remains unchanged. The other effect noted 6 hours after exposure to radiation, which may be related to the RNA effect, is an increase in the incorporation of C-14-leucine into the albuminoid-RNA complex of such lenses. Although the exact mechanism responsible for these phenomena is not known, experiments are currently in progress to attempt to extract and study this albuminoid RNA by methods which will retain most of the molecule in its intact state.

We gratefully acknowledge the technical assistance of Susan Hawes, Ruth Friel, Anna Reid, Carlos Jimenez, and Mrs. Howard Van Slyke.

REFERENCES


Discussion

Dr. Zacharias Dische, New York, N. Y. The results of Dr. Lerman's experiments indicate a faster turnover of RNA linked to albuminoid than to any other form of RNA in the time interval of a few hours after irradiation. This may point to a particular biologic significance of the albuminoid RNA. When a link between one part of the lens RNA and the albuminoid fraction had been established in our laboratory, we pointed out that the biologic significance of this fraction may be related to the fact that albuminoid seems to be deposited on the surface of the lens fibers. Although the presence of an analogous RNA fraction in other differentiated vertebrate cells could not be established at that time, there might be an analogy to the RNA formation observed by Bracket and others in the cortical zone of the fertilized amphibian oocyte. Its presence appears to be related to certain developmental stages of the oocyte. The rapid turnover of the albuminoid RNA in the fast-growing lens of the young rat, therefore, could be related to its specific biologic activity at this stage of the lens development. I believe, however, that a certain caution should be used in the interpretation of Dr. Lerman's data on the rate of incorporation of P-32. When lens homogenates are centrifuged at 600 x G, the lens capsule with its epithelium comes down together with the albuminoid. Although the total amount of RNA originating from the epithelium can, on the basis of reasonable approximate computation, form only a small part of the RNA in this sediment, the turnover of this small fraction of RNA can be very much higher than the turnover of the albuminoid RNA in the strict sense that it could account for the higher turnover of this total RNA fraction.