Histochemical changes in lens epithelium of rabbits after x-irradiation

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Acid phosphatase and ubiquinone reactions have a different distribution in the lens epithelium of the normal rabbit. Acid phosphatase is more concentrated in the germinative area than the central area, and ubiquinone is quite evenly distributed in the entire lens epithelium. Exposure to a cataractogenic dose of x-ray causes a greater decrease in the acid phosphatase in the germinative zone than in the central zone. Part of the decrease may be due to loss of cells in this area, but at least half of the loss seems to be due to a decrease in acid phosphatase in the remaining cells. This is first noted at 14 days after exposure. Ubiquinone does not show any decrease to 35 days after exposure, and a slight decrease in the germinative zone at 60 days after exposure. The difference in distribution in the control lens, and the effect of x-radiation suggest that the two systems have different functions.

Key words: lens epithelium, radiation effects, acid phosphatase, ubiquinone, distribution, enzyme histochemistry, time factors, light transmission, x-ray cataract, rabbits.

The most sensitive part of the lens epithelium of the rabbit to x-radiation is the germinative area (periphery). This has been shown in clinical and histological studies using lead shields to protect the different areas of the lens. The germinative area has the most cells and the greatest number of mitoses per unit area. Lens fibers are formed from cells which migrate from this area to the equatorial region. X-ray damage to the cells in the germinative area results in the collection of malformed fibers under the capsule, forming a posterior subcapsular cataract. The central area is also damaged by x-radiation, but to a lesser extent and with the changes becoming evident at a later time. Injury from x-radiation limited to the central zone results in damaged lens epithelial cells, but these do not migrate to form the characteristic posterior subcapsular opacity associated with x-irradiation of the germinative zone. The germinative zone is thought to be more active metabolically because of the higher incidence of cell division and cell migration to form lens fibers. The central zone is regarded as less...
active, although its function has been suggested as being important in maintaining the transparency of the lens.\textsuperscript{3}

We have used histochemical methods to study the changes in the lens epithelium. Acid phosphatase was chosen since it is generally regarded as an indicator for lysosomal enzymes.\textsuperscript{4} The ubiquinone reaction was selected because it is thought to be an important part of the electron transfer in the respiratory chain.\textsuperscript{5}

**Materials and methods**

Albino female rabbits were obtained from the same source with an average weight of 2.5 kilograms. The eyes of the rabbits were exposed alternately to 2,000 rad of x-ray; 250 Kv., 15 Ma., with one-half value layer of 0.75 ml. copper. The distance was 24.5 cm., and the cone had a diameter of 2 cm. The output was 1068 rad per minute and each eye was exposed to 2,000 rad. Exposed and control animals were killed at 1, 2, 3, 5, 7, 14, 28, 35, 42, and 60 days. The eyes were removed and placed in 7 per cent formalin at 5° C. for 20 minutes. The lenses were then removed and the capsule with epithelium was separated from the fibers with the aid of the dissecting microscope. (Lenses placed directly in formalin hardened too rapidly to permit making lens mounts.) The lens mounts were placed on a slide and divided into quarters for appropriate stains.

The acid phosphatase preparations were incubated in a mixture of one molar acetate buffer pH 5.0, 5 per cent lead nitrate, 2 per cent sodium beta-glycerophosphate, and distilled water for one hour.\textsuperscript{5} Lead phosphate was precipitated at the site of acid phosphatase activity. The slides were immersed in diluted acetic acid to stop the reaction. They were then rinsed in distilled water and stained in yellow ammonium sulfide for two minutes, rinsed again in distilled water, and mounted. The sites of phosphatase activity were marked by brown lead sulfide, which was formed from lead phosphate. Control mounts without substrate showed no precipitate.

Preparations for ubiquinone were incubated for 45 minutes in a 37° incubator with hydroquinone and Trizma buffer (pH 7.40), cobalt chloride, M.T.T. (3-[4,5-dimethyl-thiazolyl-l, 2]-2, 5-di-phenyl tetrazolium bromide) and catalase.\textsuperscript{5} The lens mounts were then rinsed and counterstained with 2 per cent chloroform washed methyl green for two minutes.

The specimens were examined with the microscope. Representative areas of the germinative and central zones were identified at 450x magnification. A Leitz 601 photoelectric meter was

![Figure 1](https://lovz.arvojournals.org)
used to determine the relative light transmission for each area, with the viewing light set at 5 volts and held constant by a voltage regulator. Three to five readings for each zone in each specimen were averaged, and the difference determined by subtracting the figure for the central zone from the figure for the germinative zone. By comparing the two zones in the same lens mount, we could be sure the differences observed were not caused by undetected variations in temperature, technique, or chemicals, as might occur with the comparison of specimens done at different times. These results were averaged for each time period and graphed. Ninety-one eyes were included in this report (Fig. 1).

Results

The lens epithelial mounts from control animals stained for acid phosphatase showed a particulate reaction in the cytoplasm, characteristically outlining the nuclear areas in the central zone. No counterstain was used. The ubiquinone reaction also produced a particulate reaction in the

![Fig. 2. The left column shows the acid phosphatase reaction and the right the ubiquinone reaction in the lens epithelium. A, Acid phosphatase; greatest concentration about 1 mm. wide in the germinative zone (4×). D, Ubiquinone; even distribution (4×). B, Acid phosphatase, germinative zone; many granules, and normal cell pattern in nuclear areas cannot be seen (450×). E, Ubiquinone, germinative zone; fewer granules, and nuclear areas are seen (450×). C, Acid phosphatase, germinative zone, 60 days after x-irradiation; marked decrease in number of granules (450×). F, Ubiquinone, germinative zone, 60 days after x-irradiation; some reduction in number of granules, but not as marked as acid phosphatase. Abnormal cell pattern is evident (450×).](image-url)
cytoplasm; methyl green was used as a nuclear counterstain. The difference in magnitude of the two reactions in the different areas in the control specimens was significant. The acid phosphatase reaction was concentrated in a 1 mm. wide band in the germinative area but was present in the central zone, whereas the ubiquinone reaction seemed to be evenly distributed in all areas (Fig. 2).

In the lens mounts exposed to x-radiation, the acid phosphatase reaction showed no microscopic changes to 7 days after exposure. At 14 days after exposure a reduction in the acid phosphatase reaction in the germinative zone was determined by measuring an increased light transmission. By 28 days, the increase in light transmission (decrease in acid phosphatase reaction) in the germinative zone was very evident on gross observation, with continued progressive decrease to 60 days after exposure.

The ubiquinone reaction was evenly distributed in the control animals between germinative and central zones, and no change in this distribution was noted in the specimens to 35 days after x-irradiation. We expected a decrease in the ubiquinone in the germinative zone by 28 days after x-irradiation because of loss of cells in this area. On microscopic examination we found the number of cells per unit area was decreased by about half in the germinative area, but the cells present were larger and spread out to fill the entire area. The cytoplasm in these cells was filled with ubiquinone-positive material, so that there was no significant decrease when compared with the central area. At 60 days, some decrease in the reaction in the germinative zone, as compared with the central zone, was noted. This decrease was not as great as seen with acid phosphatase.

Lens epithelial preparations stained with hematoxylin and eosin showed about 50 per cent loss of cells in the germinative zone by 28 days after x-irradiation. The cells still present showed marked changes in size of cells and nuclei, with fragmentation and other changes previously described. Cells with large nuclei and increased area of cytoplasm were frequently present.

Cross sections usually showed the cells in the germinative zone to be thicker than the cells in the central zone. This increase was up to 70 per cent in some specimens. A greater difference was noted in hematoxylin and eosin formalin fixed specimens, and less with frozen specimens stained for ubiquinone. Poppe reported the lens epithelium to be a single layer measuring 5 microns in the rabbit. He did not note any difference between the central and peripheral portions.

**Discussion**

The acid phosphatase reaction produced a particulate deposit in the cytoplasm of the lens epithelial cells, with the greatest concentration in a 1 mm. wide band in the germinative area. The acid phosphatase reaction is considered a marker for lysosomal enzymes, and our previous studies of beta-glucuronidase and aryl sulfatase showed the same distribution for these enzymes in the normal lens epithelium of rabbits. In our preparations, no nuclear counterstains were used.

The ubiquinone reaction showed a much more even distribution and no difference in intensity of reaction between the central and germinative zones could be seen on microscopic examination. With the light meter, a slightly decreased light transmission in the germinative zone was measured, indicating a greater density of particulate matter than present in the central zone. This could be due in part to the increase in thickness of these cells. Another factor was the counterstained nuclei (methyl green) in the ubiquinone preparations. Since the nuclei are more numerous in the germinative zone, the counterstaining would also tend to decrease light transmission in this area when compared to the central zone. Therefore, part of the difference between the central and peripheral...
zones shown in Fig. 1 for the ubiquinone reaction is due to the methyl green stained nuclei. Nuclear counterstains were not used in the acid phosphatase preparations and, therefore, did not affect the light meter readings in the two areas. The graph (Fig. 1) shows the difference between the two zones for acid phosphatase is much greater than for ubiquinone. This indicates a greater concentration of the acid phosphatase in the germinative zone. A small part of the greater concentration may be because of the slightly thicker cells in this area. This increased concentration suggests the acid phosphatase (and presumably other lysosomal enzymes) play an important role in lens fiber production, since this is the area that produces cells which migrate to the equatorial area to form lens fibers.11

Hanna and O'Brien12 used labelled cells and showed migration of cells from the germinative area to the lens bow area, but no indication that cells formed in the central zone migrated to the germinative area. The function of the cells in the central zone is more likely related to the general metabolism of the lens in maintaining transparency. This concept was supported by selective shielding during exposure to x-radiation in previous experiments.9 The even distribution of ubiquinone is further support for this concept since ubiquinone probably plays an important role in electron transfer in the respiratory chain.5

The greatest number of mitoses occur in the germinative area. After x-irradiation, mitosis stops within hours for 3 to 7 days, and then starts again with a rapidly occurring increase before falling to a normal rate.9 Neither the acid phosphatase or ubiquinone reaction showed any significant change coincident with the mitotic rate change, and no localized changes were seen which could have corresponded to either normal or abnormal mitotic figures.

X-radiation affects the two enzyme systems differently. The acid phosphatase reaction shows a relatively selective decrease in the germinative zone first measured at 14 days after exposure, and this is progressive through 60 days after exposure. The decrease parallels the cell depletion also present in the germinative zone, but is greater than can be accounted for on the basis of cell loss alone. In hematoxylin and eosin preparations, at 28 days after exposure to x-ray about 50 per cent of the cells in the germinative zone are gone.8 There are still twice as many cells per unit area as present in the central zone. The remaining cells vary in size but in general are larger, and fill the area. The light transmission at 28 days was greater in the germinative zone than in the central zone, indicating less acid phosphatase reaction there than in the central zone. The decrease could not be explained on the basis of cell loss alone, and part of the loss was due to loss of acid phosphatase in the individual cells. The decrease in the germinative zone continued to 60 days post exposure, the termination of the experiment. The decrease in acid phosphatase was significant at the p < 0.05 level.

The ubiquinone reaction did not change after x-irradiation. The light transmission determinations were not different to a significant extent to 35 days after exposure to x-ray. Although the cell loss was 50 per cent in the germinative zone at 28 days after exposure, there was no significant difference in the light transmission. The cells present in the germinative zone at this time have larger nuclei and a greater quantity of cytoplasm to take up the space left by the cell loss. These cells seem to maintain the ubiquinone in the cytoplasm so that the total amount remains about the same. This finding supports the concept that decrease in acid phosphatase is largely due to loss of particles in the individual cells, rather than cell depletion. At 60 days after exposure, the ubiquinone reaction was slightly decreased in the germinative zone, paralleling the decrease of the acid phosphatase.

This is supported by the work of Goldfeder and Selig.13 They showed that exposures up to 10,000 r failed to induce signifi-
cant effect on the mitochondrial oxidative metabolism of tumors. They speculated that the high resistance of oxidative metabolic events may be explained by an abundance of oxidative enzymes, so that if only a portion were destroyed the rest might be able to maintain metabolic performance. Our cytochemical stains do not show depletion of ubiquinone, which seems to be a redox compound. Others have identified ubiquinone as an important part of electron transfer in the respiratory chain.5

The ubiquinone reaction is that used by Tranzer and Pearse.7 In the retina, there is considerable doubt as to the substance or substances identified by this method. There seems to be little doubt that a redox compound is involved. In the lens, there is no evidence that the substance is not ubiquinone as originally suggested by Tranzer and Pearse.

X-ray cataracts have two stages. The first is the accumulation of abnormal cells under the posterior capsule, forming the typical posterior subcapsular cataract. If the damage is not too severe, either because the dosage of x-ray is small or at least one-fourth of the lens epithelium is protected by a lead shield, the lens does not become completely opaque; eventually normal fibers are again produced and the only residual is the accumulation of these opaque cells. If the damage is too great, at 4 to 6 months after exposure in rabbits, the entire lens becomes opaque. This second stage coincides with complete disruption of the entire lens epithelium, whereas the first is correlated with the damage noted in the germinative zone.

The effect of x-radiation on acid phosphatase suggests this is an important aspect of x-ray damage to developing lens fibers, as in the first stage of cataract formation. The relative resistance of ubiquinone to x-irradiation suggests that this is important in preventing the complete cataract formation in the second stage. Our present experiments are designed to study these concepts more completely.

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REFERENCES