The effect of X-irradiation on Na-K ATPase and cation distribution in rabbit lens

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The Na-K ATPase activity of rabbit lens was measured at various times after exposure to a single dose of 2000 rads of X-ray and was compared with that in contralateral control eyes. A decrease in enzyme activity in both whole lens and in isolated capsule-epithelium was first observed 3 to 4 weeks after irradiation and became increasingly marked at 7.5 weeks after X-ray. These findings are consistent with our earlier observations that active transport of cations is reduced in these lenses and support the view that loss of membrane ATPase is responsible for the impairment of the cation pump in X-irradiated lenses. Despite a significant loss of the enzyme, X-irradiated lenses were able to maintain near normal levels of total cations (Na\(^+\) + K\(^+\)), thus accounting for their normal hydration. The results of the changes in lens Na\(^+\) and K\(^+\) levels revealed that between 4 and 7.5 weeks after X-ray, the gain in Na\(^+\) was compensated by an equivalent loss of K\(^+\). A breakdown of this relationship of 1:1 exchange of Na\(^+\) for K\(^+\) is accompanied by a disproportionate increase in Na\(^+\) and water. (INVEST OPHTHALMOL VIS SCI 22:180-185, 1982.)

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A number of previous studies have shown that exposure of the lens to excessive ionizing radiation brings about an increase in permeability.\(^1\),\(^2\) The altered permeability is believed to be the contributing factor in lens hydration, which is implicated in the development of a number of experimentally induced cataracts.\(^3\)

Recent studies from this laboratory\(^4\) have demonstrated that X-irradiation of rabbit lens with a single dose of 2000 rads results in not only an increase in permeability but also a decrease in the active transport of rubidium ion. The results lead to the conclusion that impairment of active transport may be due to the inactivation of Na-K-activated ATPase. We also observed that despite dramatic changes in permeability and active transport of cations, the X-rayed lenses were able to maintain near-normal hydration for several weeks until the development of mature cataracts. These studies suggested that the constancy of hydration may have been due to mechanisms by which Na\(^+\) gain in the lens was compensated by an equivalent loss of K\(^+\).

The purpose of this investigation is to provide evidence for the inactivation of Na-K ATPase after X-irradiation and to examine the distribution of the cations, Na\(^+\) and K\(^+\), in the lens as a function of time after exposure to X-ray.
Methods

Albino rabbits, 5 to 6 weeks of age and weighing 0.6 to 1.0 kg, were used. One eye of each rabbit was exposed to an air dose of 2000 rads of X-rays from a 85 kvp source through a 0.5 mm aluminum (built-in) filter. The radiation parameters were 5 mA, 150 rads/min, and a target distance of 16.5 cm between the source and the center of the lens. The second eye served as control, which received less than 50 rads. During irradiation, the animals were under pentobarbital anesthesia (35 mg/kg injected into the thoracic cavity). The details of the shielding around the X-rayed eye and the estimation of the radiation received by the contralateral control eye have been described earlier.4

The animals were killed by air embolism at various intervals after exposure to X-ray, and the lenses were removed and examined under a microscope. Except for posterior subcapsular haziness, X-rayed lenses appeared clear until 8 to 9 weeks after exposure when mature cataracts with complete opacity and swelling suddenly developed in 90% of the animals. The corresponding contralateral controls remained clear.

The activity of Na-K-activated ATPase in whole lens or in capsule-epithelium (CE) was measured by the procedure described by Bonting et al.,5 except that homogenates were assayed immediately without lyophilization. The CE from each lens was removed by making a small incision in the equatorial region and carefully peeling off the entire capsule with the attached layer of epithelium, which weighed between 4 and 6 mg, depending on the age of the animal.

The tissues were homogenized in 0.23M Tris buffer, pH 7.5, containing 0.75 mM ethylene glycol tetraacetic acid (EGTA): 2 ml for whole lens and 0.7 ml for CE were used. ATPase was assayed by adding aliquots of homogenates to a reaction mixture consisting of 2 mM ATP, 2 mM Mg++, 5 mM K+, 58 mM Na+, 10 mM CN− and 0.1 mM ouabain and incubating for 1 hr at 37° C. The reaction was stopped by the addition of 50% trichloroacetic acid (TCA) and the amount of phosphate was determined in the supernatant. The activity of Na-K ATPase was calculated as the difference of activities obtained in the presence and absence of ouabain.

Hydration of the lenses was determined by drying them to constant weight at 100° C. The dried lenses were homogenized in 10% TCA, the mixture was centrifuged, and the supernatant was used for the assay of Na+ and K+ ions by flame emission, using an atomic absorption spectrophotometer (Perkin-Elmer Model 270).

Results

Fig. 1 summarizes the data for wet and dry weights as well as hydration of X-rayed lenses compared with contralateral controls as a function of time after irradiation. Until about 7.5 weeks after exposure to X-ray the wet weight of the control lenses was consistently higher than that of the X-rayed lenses. However, at the time of mature cataract development (8 to 9 weeks) the wet weight of X-rayed lenses was nearly 60 mg higher than the control weight. On the other hand, the dry weight of the X-rayed lenses at the time of cataract development was only 50% of that of the contralateral control, reflecting a considerable loss of proteins. The hydration of both the X-rayed and control lenses for the first 7.5 weeks after exposure was essentially the same. Coincident with the appearance of mature cataract (total opacification) the hydra-
Fig. 2. Effect of X-irradiation on Na-K ATPase activities in rabbit whole lens (upper panel) and CE (lower panel). The results are expressed as means ± S.D. for at least five (whole lens) and seven (CE) experiments.

The first significant decrease (37%) in Na-K ATPase in X-rayed lenses was observed at 3 weeks after exposure (Fig. 2, top panel). Between 4 and 7.5 weeks after irradiation the activity of the enzyme in X-rayed lenses was reduced to approximately 60% of that in the control. At the time of the appearance of cataract (8 to 9 weeks after X-ray), the enzyme activity was further reduced and was only 20% of that in the control lens. Since the enzyme activity is expressed on the basis of wet weight and there is significant hydration in the cataractous lens, the loss in Na-K ATPase activity between 7.5 weeks and 8 to 9 weeks after X-ray may not have been as great as it appeared.

Since membrane ATPase in the lens was heavily concentrated in the epithelium, which is the primary site of active transport, it was considered meaningful to also measure the effect of X-ray on the activity of the enzyme in this region of the lens (Fig. 2, lower panel). The activity of the enzyme, expressed as micromoles of phosphorus liberated per hour per single CE from X-rayed lens, was reduced by 40% at 4 weeks and by 72% at 7.5 weeks after X-ray. A gradual increase observed in the activity of the enzyme in the CE of control lenses was apparently related to the increase in the size of the lens during the experimental period.

From the data in Fig. 2 it can be calculated that the decrease in Na-K ATPase activity in the whole lens was due to a drop in the activity of the enzyme in both the CE and the fiber mass. For example, 7 weeks after X-ray the enzyme activity in CE was lowered by 0.4 μmoles of P per CE per hour, whereas the corresponding decrease in the whole lens was 0.96 [(control lens weight in grams × μmoles of P per gram per hour) − (X-rayed lens weight in grams × μmoles of P per gram per hour)]; (0.324 × 5.2) − (0.276 × 2.6)]. Thus the decrease in Na-K ATPase in CE of X-rayed lens accounted for 42% of the total loss of activity in the whole lens.

The finding in our previous study that despite significant changes in active transport of cations and permeability of lens membranes, X-rayed lenses were able to maintain normal hydration for several weeks suggested the possibility that total cation content may have remained constant during this period. To show that constancy of total cation levels in X-rayed lens is responsible for the normal hydration, the levels of both Na⁺ and K⁺ were determined in X-rayed lens and compared with contralateral controls.

As shown in Table I, the total amount of Na⁺ + K⁺ in the X-rayed lenses 3 to 7.5 weeks after irradiation ranged from 25.9 to 30.5 μEq/lens. The corresponding values for the contralateral controls ranged from 25.5 to 33.5 μEq/lens. The slightly higher cation content in the control lenses may be due to their higher growth rate compared to the lenses exposed to X-ray. However at 8 to 9 weeks after irradiation when mature cataracts develop, the total cation content of these lenses increased to 47.6 μEq compared with 27.2 in the controls. Thus hydration of
X-rayed lenses remained near normal as long as the total cation content remained within normal limits.

**Discussion**

Previous studies have demonstrated that there is an increase in lens permeability and hydration after exposure to X-ray. In an earlier study from this laboratory we showed that X-irradiation not only results in increased permeability of lens membranes but leads to an impairment of active transport of rubidium ion. The decrease in cation pump activity was first observed 4 weeks after exposure to X-ray and became increasingly marked at 7.5 weeks or just prior to the appearance of complete lens opacities. It is clear from the results of this study that decreased Na-K ATPase activity in X-rayed lens corresponds well with the previously observed inhibition of the cation pump. The measurement of enzyme activity in CE, which is the major transport site, confirms the conclusions arrived at from the data on whole lens (i.e., that X-irradiation leads to a loss in the activity of transport ATPase) and supports the view that diminished activity of this enzyme is responsible for the reduction of active transport of cations in these lenses.

The present finding that X-irradiation leads to a decrease in membrane-bound ATPase in rabbit lens is in contrast to the studies of Palva, who found no loss of biochemically measured Na-K ATPase in isolated epithelium and capsule preparations from control and X-irradiated rat lenses from 3 to 90 days after irradiation. It is not known whether the contrasting findings are due to the species difference or to the fact that a lower X-ray dose (1500 rad) was employed in the previous study.

Although this is the first report that demonstrates the loss of Na-K ATPase activity in X-rayed lens, decreased activity of this enzyme has been observed in sugar cataracts and in the hereditary cataracts of the Nakano mouse. In the lenses of galactose-fed animals a pronounced decrease in the activity of the enzyme occurred in the fibers prior to the formation of nuclear cataracts while the drop in enzyme level of capsule plus epithelium was noted only in mature cataracts.

Although the exact mechanism for the inactivation of Na-K ATPase in X-rayed lenses is not known and is a matter for speculation, it seems unlikely that the observed decrease in the activity of the enzyme is due to the direct effect of radiation, since there was no significant change in enzyme activity at 2 weeks after X-ray and the decrease in activity was manifest only at 3 to 4 weeks after irradiation. It is of interest that the activities of a number of other sulfhydryl enzymes in the lens are also lowered after exposure to X-ray. However, the magnitude of the reduction in activity of Na-K ATPase (50% to 70%) is far greater than that noted for glucose-6-phosphate dehydrogenase, hexokinase, glyceraldehyde phosphate dehydrogenase, and phosphofructokinase, the activities of which were lowered by about 25% compared with the contralateral controls.

Another possible mechanism for inactivation of the enzyme may be due to the deple-
tion of glutathione (GSH) in the X-rayed lens. It has been suggested that GSH may act as a protective agent for -SH groups of membrane ATPase and that loss of GSH may lead to disulfide bond formation and thus contribute to the inactivation of the enzyme through a conformation change. It was therefore of interest to look for a correlation between the levels of GSH and Na-K ATPase in the X-rayed lens. A dramatic decrease in the level of GSH in X-irradiated lenses was found as early as 1 week after exposure to X-ray; at the end of 3 weeks, the GSH content in the whole lens decreased by 30%, and a 75% loss occurred at 7.5 weeks.4 Thus the decrease in Na-K ATPase activities in the whole lens observed between 3 and 7.5 weeks after X-ray appears to accompany the loss of GSH. In contrast to the whole lens, however, GSH in the epithelium was found to decrease by only 20% at 4 weeks and 40% at 7.5 weeks after X-ray appears to accompany the loss of GSH. In comparison to the epithelium, however, GSH in the epithelium was found to decrease by only 20% at 4 weeks and 40% at 7.5 weeks after X-ray compared to the corresponding values for enzyme inactivation of 40% and 72%, respectively. This lack of strict stoichiometric relationship between the level of GSH and the activity of Na-K ATPase does not necessarily rule out a role for GSH in maintaining membrane ATPase activity in the lens, but it does raise the question of other possible mechanisms for the inactivation of transport ATPase in X-rayed lens.

The observation that the total cation level (Na⁺ + K⁺) in the lens remains constant until 7.5 weeks after irradiation provides an explanation for the constancy of lens hydration during the same period. This is achieved by the ability of the lenses to compensate the gain in Na⁺ by an equivalent loss of K⁺ despite the fact that the Na-K ATPase in the epithelium is reduced by 40% to 70%. The ability of the lens to maintain normal hydration for a limited period in spite of reduced levels of Na-K ATPase has been observed previously. Kinoshita⁴ has shown that ouabain-treated lenses in which 50% of transport ATPase was inhibited maintained normal hydration as long as the exchange of Na⁺ for K⁺ was 1:1. It is apparent from the present findings (Fig. 3) that between 4 and 7.5 weeks after X-ray, the change in lens Na⁺ is nearly equal to that of K⁺. This relationship of 1:1 exchange of Na⁺ for K⁺ ion apparently breaks down just prior to the appearance of mature cataract, when there is a disproportionate increase of Na⁺ and water coincident with total opacification.

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REFERENCES
1. von Sallmann L and Locke BD: Experimental studies on early lens changes after Roentgen irradiation.
II. Exchange and penetration of radioactive indicators (Na\textsuperscript{45}, K\textsuperscript{42}, I\textsuperscript{131}, P\textsuperscript{32}) in normal and irradiated lenses of rabbits. Arch Ophthalmol 43:431, 1951.


