with NAD$^+$ as the cofactor in these cytosolic fractions, a result in agreement with our study on this enzyme in rat retina.$^7$

This investigation has shown that the capsule-epithelium has high activities of three cytosolic NADPH-producing enzymes. While the high activity of G-6-PD has been reported,$^9$ the present work provides the first evidence that the NADP$^+$-dependent isocitrate dehydrogenase and malic enzyme are also present at high activities in the epithelial cells of the lens. This is a very interesting finding, particularly since the avascular lens is often considered an “anaerobic” tissue, despite the presence of mitochondria in the epithelial cells. Assessing the physiological function of isocitrate dehydrogenase and malic enzyme will require additional experiments, since these in vitro measurements of enzyme activities do not necessarily reflect metabolic processes in the lens under limiting oxygen concentrations and oxidative stress. Nevertheless, the presence of these NADPH-producing enzymes raises the possibility that mitochondrial activity during oxidative stress may be more important than previously recognized in the regulation of the intracellular content of GSH, generating malate and citrate as a source of isocitrate in the cytosol. In this regard, it is of interest that mitochondrial glucose oxidation, as measured by the rate of production of $^{14}$CO$_2$ from C-6-labeled glucose, is increased significantly in lenses exposed to t-butyl-hydroperoxide, an oxidant for GSH.$^5$

Key words: lens, capsule-lens epithelium, rabbit, NADP$^+$-dependent isocitrate dehydrogenase, NADP$^+$-dependent malic enzyme, glucose-6-phosphate dehydrogenase

From the Eye Research Institute of Oakland University, Rochester, Michigan. Supported by grant EY-05100 from the National Eye Institute, National Institutes of Health and by a Biomedical Research Support Grant (BRSG) to Oakland University. Submitted for publication: June 24, 1987; accepted December 7, 1987. Reprint requests: Dr. Barry S. Winkler, Eye Research Institute of Oakland University, Rochester, MI 48309.

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A Raman Study of Disulfide and Sulfhydryl in the Emory Mouse Cataract

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Emory mice (EM) are genetically predisposed to late-onset cataract formation. Our early work has shown UV-exposure slightly enhanced the expected 2 SH $\rightarrow$ SS conversion of normal mouse lenses only in the cortical regions. There was essentially no difference in the disulfide profiles of the nuclear region between UV-exposed and control lenses. Since the first noticeable change in the Emory mouse is a hazy nucleus when a lens is examined in vivo, we wondered if cataractogenesis in this model is different from the UV-produced cataract. This question was answered by comparing the visual axis profiles for SH and SS in early EM cataracts and in clear lenses from age-matched controls. The sulfhydryl profiles show that the SH level of 8.5-month-old EM lenses is essentially the same as that of the controls. Likewise, the disulfide profiles show a significant difference. The results clearly demonstrate that EM lenses do not undergo accelerated disulfide production. Therefore for the EM lens, the early stage of cataract formation must involve factors other than just accelerated oxidation of protein SH or glutathione SH. Invest Ophthalmol Vis Sci 29:823–826, 1988

We have previously presented evidence that the aging mouse lens is characterized by an apparent conversion of nuclear SH to S-S, a conversion which is accelerated in the cortex by prolonged exposure to long wave ultraviolet light in vivo.$^1$ This UV effect and other results support a hypothesis that cataractogenesis in the Emory mouse may, to a significant
extent, involve oxidation of SH. This paper reports the results of Raman measurements of SH and S-S in lenses of 9-month-old Emory mice with definite onset of cataractogenesis as compared with age-matched clear control lenses. The advantages of Raman spectrometry as a noninvasive, nondestructive microprobe of the intact living lens have been noted before. In this work, a particular advantage has been the use of the phenylalanine signal for normalization of the data from different lenses.

Materials and Methods. The laser Raman system employed in this study was the same as that described previously. ² The X-Y translation stage, as also described earlier, ³ was used for careful optical dissection of the visual axes of the lenses studied. By placing an opaque paper matting over the spectrometer slit, the portion of the visual axis image examined could be limited to only 1.5 mm at a time. This corresponds to a 200 µm segment in the lens. Each visual axis was divided into 21 spectra by using the micrometer stage to move the sample cuvette relative to the entrance slit. A CR-6 Argon ion laser was used to produce the 514.5 nm (green) excitation line for the experiments. A tunable laser filter was employed to remove plasma lines from the excitation line before it entered the sample. The average laser power used was 190 mW. The slit-width varied between 300 and 350 µm, depending on the individual sample, which is equivalent to approximately a 7 wavenumber spectral slit-width resolution.

Both the Emory and the control mice were raised under standard colony conditions, and their treatment was in accordance with the ARVO Resolution on the Use of Animals in Research. A posterior approach was used to remove the lenses from the freshly dissected eyeballs. The EM lenses were selected to be of the proper age to exhibit early changes (Grade 1 of 5), ⁴ which would not be severe enough to interfere with Raman spectroscopy; a control lens was age-matched with each of the EM lenses. The lenses were maintained throughout the Raman experiment in a nutritive saline solution. The mice studied ranged in age between 6 to 9 months old. The Emory lenses were all visually fairly clear, however, slit-lamp examination did reveal pinhead opacities in a majority of the samples. This is an important consideration in order to examine the visual axis of the whole lens, which is essentially only possible for Grade 1 Emory cataracts. The anterior of each lens in the study was placed on the bottom of the quartz cuvette. Even though some of the EM lenses had small opacities, the laser beam was able to penetrate along the entire visual axis as judged by the unbroken, uniform straight line visible as the image of the visual axis. This type of image would not have been seen if the laser beam had been significantly scattered due to the presence of pinhead opacities.

Results. Figure 1 contains a representative Raman scan of the 400–700 cm⁻¹ region (S-S) of the central
nuclear region of an 8.5-month-old EM lens. Similarly, Figure 2 contains a representative scan of the 2500–2800 cm⁻¹ region of a 6-month-old EM lens. The large, broad line at 508 cm⁻¹ represents the disulfide stretching mode. The sharp line at 624 cm⁻¹ represents phenylalanine, and was used as the reference signal in normalizing the disulfide intensity since Phe is a marker for protein concentration in the lens. Likewise, the broad peak at 2580 cm⁻¹ is due to sulfhydryl (SH) and is normalized by the protein reference line at 2731 cm⁻¹. Twenty-one spectra were collected for each lens analyzed, and the ratios of S-S/Phe (508/624 cm⁻¹) and SH/protein (2580/2731 cm⁻¹) were determined for each point. The intensity ratios from both spectral regions versus visual axis length for representative lenses are plotted in the graphs in Figures 3 and 4. In both graphs, the EM lenses show more scatter than the control lenses, but this is to be expected because some of the EM lenses were already undergoing cataractogenesis at the time of Raman analysis, whereas all the control lenses were clear to the unaided eye and also by slit-lamp inspection. It is obvious in Figure 3 that the disulfide profiles are slightly offset from each other. This is believed to be due to the fact that the geometric center of the lens (which is plotted) is not necessarily the metabolic center of the lens. Therefore, there is lens-to-lens variation in the exact location of where the densest packing (ie, greatest protein concentration) of the oldest lens cells lies. In spite of this geometrical difference, it is easy to see that the disulfide profiles of the EM and control lenses do not significantly differ for lenses of approximately the same age. In Figure 4 it is also plainly seen that the sulfhydryl profiles of age-matched EM and control lenses do not differ significantly in their maxima and the EM minima are consistently slightly greater than the control minima. Therefore, the data do not support the hypothesis that 2 SH → S-S conversion occurs more rapidly than normal in the Emory mouse, a strain genetically predisposed to late-onset cataract formation.

Discussion. Although considerable work has been reported for the EM cataract,4–6 there is still no firm evidence for a mechanism of its formation, unlike the Nakano mouse, for instance, where the lens early in life begins to accumulate an abnormal polypeptide which is an inhibitor of Na,K-ATPase.7 Knowledge of such a mechanism would be of interest not only as a possible explanation for some types of human senile cataract, but also as a rational way to choose drugs.
which may retard cataract formation. Our earlier finding that ultraviolet light exposure resulted in an increase in cortical S-S certainly suggests that oxidation could be a factor in the EM cataract.\(^1\) Other previous studies\(^4\) of protein sulfhydryl levels found there was indeed a significant decrease in soluble PSH levels of 15- to 20-month-old opaque EM lenses as compared to clear control lenses. It was therefore of interest to determine whether an acceleration of SH to S-S is occurring even in the cataract initiation stages of the EM lens, rather than as an accompanying phenomenon of the developed cataract. Our results clearly show that such an effect is not detectable by our procedure. Raman spectroscopy is sensitive enough to detect differences in disulfide concentration levels of 5-10%, depending upon the clarity of the lens. However, the true beauty of the technique lies in the fact that the disulfide concentration measured is that actually present in the natural milieu of the intact lens, and is not distorted by concerns for artificial disulfide production inherent to homogenized lens assays. It is still possible, though, that oxidation at the fiber membrane could have a marked effect on membrane viability without a measurable effect on the total SH concentration measured by the Raman spectrometer. It is also possible that the underlying defect in the EM lens has an effect which is independent of the UV effect but is still additive. It is well known that unrelated metabolic insults to the lens may together produce cataract even when either insult alone is below the critical threshold.\(^8\) The noteworthy observation reported here is that disulfide production and sulfhydryl loss is not occurring at an accelerated pace in the initiation of the EM cataract. This conclusion should not be entirely unexpected because an old, perfectly clear mouse lens shows a marked accumulation of S-S in the nucleus.\(^9\) This phenomenon occurs also in the rat, but not in guinea pig or man, and appears to be correlated with the development of a hard nucleus in mouse and rat lenses as contrasted with the soft nucleus in guinea pig and normal human lenses. It has been shown\(^10\) that in a hereditary cataract of the rat there is a marked conversion of SH to S-S in the lens protein, with accompanying protein insolubilization. These changes did not appear in the 60-day-old lenses, but were significant in 95-day-old lenses. This phenomenon, the oxidation of PSH to PSSP and the accumulation of the product in the insoluble protein fraction, also occurs in the human lens which has developed senile cataract.

**Key words:** disulfide, Emory mouse, Raman spectroscopy, sulfhydryl, lens

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