the ciliary body of rabbits. This isolation procedure does not require the use of enzymes and may therefore be useful for providing substantially pure fractions of nonpigmented cell membranes with minimal loss of activity of receptors and other membrane proteins for pharmacological and biochemical investigations. The nonpigmented cell layer isolated by our procedure may also be dissociated to form cell suspensions containing greater than 99.8% nonpigmented cells in neonates and greater than 98.5% nonpigmented cells in young adult tissue. A great majority of the nonpigmented cells in these suspensions appear to be viable by dye exclusion. Furthermore, cells in these suspensions appear to remain viable for several days in tissue culture medium and in some cases have been observed to attach to the substrate and form clusters of spreading cells with minimal contamination from pigmented cells. Our isolation procedure may therefore also be useful as a starting point for tissue culture, immunocytochemical studies and the investigation of ion transport.

Key words: ciliary body epithelium, nonpigmented ciliary body epithelium, cell separation techniques, glaucoma, cell culture

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High Activities of NADP+-Dependent Isocitrate Dehydrogenase and Malic Enzyme in Rabbit Lens Epithelial Cells

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Measurements were made of the activities of the NADP+-dependent isocitrate dehydrogenase, malic enzyme and glucose-6-phosphate dehydrogenase in cytosolic supernatants of whole lens and capsule-epithelium. The activities of all three NADP+-dependent enzymes were concentrated in the capsule-epithelium relative to the activities measured in the whole lens. These results show for the first time that the rabbit lens epithelial cells contain substantial activities of the NADP+-dependent isocitrate dehydrogenase and malic enzyme. It is proposed that these enzymes together with...
glucose-6-phosphate dehydrogenase are involved in the regulation of the intracellular level of glutathione during oxidative stress by providing the NADPH necessary for the activity of glutathione reductase. Invest Ophthalmol Vis Sci 29:821–823, 1988

Glutathione (GSH) is present in the lens in high concentration and plays an important role in defending against oxidative damage. This function of GSH is coupled to pathways that produce NADPH which is required for the reduction of oxidized glutathione by glutathione reductase. The hexose monophosphate shunt (and glucose-6-phosphate dehydrogenase, EC 1.1.1.49) has received the most attention as the enzymatic pathway which produces NADPH. Indeed, it has been shown for many tissues, including cornea, lens and retina that the activity of the shunt pathway is stimulated when challenged by a GSH oxidant. Recently, our laboratory has provided evidence that the retina contains several additional NADPH-producing enzymatic pathways which participate in the maintenance of the level of GSH. These include the NADP+-dependent isocitrate dehydrogenase (EC 1.1.1.42) which catalyzes the oxidative decarboxylation of isocitrate to a-ketoglutarate and the NADP+-dependent malic enzyme (EC 1.1.1.40) which oxidatively decarboxylates malate to pyruvate. Because of the strong interest in oxidative processes in the ocular lens, measurements were made of the activities of these two NADP+-dependent enzymes as well as of the activity of glucose-6-phosphate dehydrogenase (G-6-PD) in whole lens and in a separated fraction containing capsule-lens epithelium.

**Materials and Methods.** Albino rabbits at about 12 weeks of age and weighing between 2.0 and 2.5 kg were used in accordance with the ARVO Resolution on the Use of Animals in Research. Following sacrifice of the animals with an overdose of sodium pentobarbital, the eyes were removed and the lens was isolated from other ocular structures. Capsule and lens epithelium were removed by making a small incision at the equator and carefully peeling off the entire capsule with attached epithelial layer. Thus, enzyme activities were measured in whole lens and in capsule-lens epithelium. A single whole lens or a single capsule-lens epithelium was homogenized in 1.0 ml of the appropriate buffer. Cytosolic extracts were prepared by centrifugation of the homogenates for 20 min at 20,000 g and collection of the supernatants. Assay mixtures were in a final volume of 1.0 ml, using typically 0.1 ml supernatant. The activities of the NADP+-isocitrate dehydrogenase and the NADP+-dependent malic enzyme were assayed as described previously and G-6-PD was assayed according to Bergmeyer. Activities were determined by measuring the change in absorbance of NADPH at 340 nm. All reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Protein was determined according to the BCA procedure, Pierce Chemical Co. (Rockford, IL). The averaged wet weight of the whole lens used in this study was 305 mg and the averaged soluble protein was 85 mg. For the capsule epithelium, wet weight was, on average, 3.7 mg and soluble protein was 0.1 mg.

**Results and Discussion.** Table 1 shows the activities of the three NADP+-dependent cytosolic enzymes, G-6-PD, isocitrate dehydrogenase and malic enzyme. It is evident that the capsule-epithelium has much higher specific enzyme activities (units/g protein) than does the whole lens. In the case of G-6-PD, its activity in the capsule-epithelium amounts to 29 units/g protein, a value about 45-fold higher than that found in whole lens. The activity and regional concentration of G-6-PD in the capsule-epithelium are in good agreement with the findings of Giblin and Reddy. The major new finding of this study is that the capsule-epithelium also contains high activities (units/g protein) of the NADP+-dependent isocitrate dehydrogenase and the NADP+-dependent malic enzyme. The activity of isocitrate dehydrogenase in the capsule-epithelium, eg, 27 units/g protein, is similar to the activity of G-6-PD, while the activity of the malic enzyme, eg, 18 units/g protein, is about one-third lower than the activities of the other two enzymes. The activities of NADP+-dependent isocitrate dehydrogenase and malic enzyme in capsule-epithelium were 53- and 62-fold higher, respectively, than in whole lens. Isocitrate dehydrogenase did not work

### Table 1. NADP+-dependent enzyme activities in rabbit whole lens and capsule-epithelium

|                        | Whole lens |  | Capsule-epithelium (CE) |
|------------------------|------------|-----------------------------|
|                        | units/lens | units/g protein              | units/CE | units/g protein |
| G-6-PD                 | 0.055 ± 0.007 (7) | 0.640 ± 0.077 (7) | 0.0029 ± 0.001 (8) | 29 ± 7 (8) |
| Isocitrate dehydrogenase | 0.043 ± 0.005 (7) | 0.509 ± 0.073 (7) | 0.0027 ± 0.001 (8) | 27 ± 8 (8) |
| Malic enzyme           | 0.025 ± 0.003 (7) | 0.290 ± 0.040 (7) | 0.0018 ± 0.001 (7) | 18 ± 6 (7) |

* One enzyme unit corresponds to the conversion of 1 micromole of substrate per minute at 25°C under optimal conditions. Results are expressed as mean ± SD and the number of experiments is indicated in parenthesis. G-6-PD = glucose-6-phosphate dehydrogenase.
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.$^3$

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

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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 vitro, 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 no 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