The sorbitol pathway in the human lens: aldose reductase and polyol dehydrogenase

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The sorbitol pathway in human lenses is evaluated on the enzymic level. Adult lenses, normal and nondiabetic as well as diabetic cataracts, are found to contain limited levels of aldose reductase (AR) and high levels of polyol dehydrogenase (PD) relative to the animal lens. AR is confined primarily to the lens epithelium and is two to three times higher in juvenile lenses than in the adult lens. The level of AR in the epithelium of juvenile lenses is sufficient to cause significant osmotic stress. The $K_m$ of glucose for AR is roughly 200 mM, whereas the $K_m$ for NADPH is 0.06 mM. NADP inhibits human lens AR noncompetitively and has a $K_i$ equivalent to the $K_m$ for NADPH. PD occurs in both the lens epithelium and cortex, remains persistently high with age, and decreases with increased cortical involvement. The $K_m$ of sorbitol for PD is 1.4 mM and for NAD is 0.06 mM. NADH ($K_i$ 0.002 mM) competitively inhibits PD in the forward direction. PD purified 100-fold from diabetic and nondiabetic cataracts and normal lenses exhibit similar kinetic constants. PD has an extremely high $V_max$ in the fructose-to-sorbitol direction. The $K_m$ of fructose is 40 mM and for NADH is 0.02 mM. At high enough concentration, alrestatin also inhibits PD. The added activities of AR and PD in producing sorbitol and fructose in combination with decreased hexokinase with age may account for diabetic cataract formation in human lenses exposed to a high glucose stress. Nucleotide levels are reported for senile cataractous lenses.

Key words: sorbitol pathway, aldose reductase, polyol dehydrogenase, distributions, kinetics, nucleotide levels

The enzymes aldose reductase (AR) and polyol dehydrogenase (PD) constitute the sorbitol pathway. Although it is well established that the sorbitol pathway leads to sugar cataract in animal lenses,1, 2 its role in the etiology of diabetic cataracts in human lenses is uncertain.

In the animal lens, a higher specific activity of AR relative to hexokinase (HK) and PD, sufficient regeneration of NADPH (the co-factor for AR), and levels of NADH adequate to inhibit PD promote the accumulation of sorbitol from glucose.3-6 The concentration of sorbitol formed under these circumstances is substantial enough to lead to osmotic stress and cataract formation.7, 8

Uncertainty regarding the contribution of
the sorbitol pathway to diabetic cataract formation in the human lens arises from conflicting studies on the levels of polyol (sorbitol and fructose) in freshly excised and in incubated diabetic and nondiabetic lenses and from inconsistent and limited data regarding the enzyme levels and regulatory properties of human lens AR and PD. Pirie and van Heyningen\textsuperscript{9} and Pirie\textsuperscript{10} made the initial suggestion that the sorbitol pathway plays a deleterious role in the human diabetic lens. They reported that lenses from nondiabetic subjects contain no sorbitol and little fructose whereas lenses from diabetic patients contain 1 to 4 \( \mu \text{mol/gm polyol.} \) Conversely, Kuck\textsuperscript{11} found that nondiabetic human lenses contain both sorbitol and fructose. In agreement with Kuck, Heaf and Galton\textsuperscript{12} and Pfaffenberger et al.\textsuperscript{13} reported trace amounts of sorbitol and fructose in both diabetic and nondiabetic lenses. Undoubtedly, differences in the severity of cataract, state of integrity of the lens epithelium, blood sugar levels of the diabetic subjects, and enzyme levels of AR and PD affect the absolute amount of polyol reported. It is quite noteworthy that Varma et al.\textsuperscript{14} recently reported a significant increase in sorbitol and fructose in lenses of diabetic patients with extremely elevated blood sugar levels. Pirie and van Heyningen\textsuperscript{9} found a similar situation to occur in one 35-year-old diabetic subject with a blood sugar level of 484 mg/100 ml.

Incubation studies by Varma et al.\textsuperscript{14} and Chylack et al.\textsuperscript{15} have shown that the adult human lens, clear or cataractous, is capable, at an increased glucose load of 35.5 mM, of producing 1 \( \mu \text{mol of sorbitol and fructose per lens in 24 hr.} \) Although these results indicate that the sorbitol pathway is operable in the human lens, the amount of polyol detected by the investigators is insufficient on a whole lens basis to exert osmotic stress. The absolute concentration of polyol detected may again reflect either the integrity of the lens membrane or the enzymic activities of the sorbitol pathway.

An evaluation of the sorbitol pathway in human lenses has not been performed on the enzymic level. Although Pottinger,\textsuperscript{16} Chylack et al.,\textsuperscript{15} and Friedburg\textsuperscript{17, 18} have reported lower levels of AR in human lenses than those found for animal lenses, the values reported for AR activity are widely deviant. The high levels of PD relative to AR reported by Friedburg\textsuperscript{17, 18} and Chylack et al.\textsuperscript{15} in nondiabetic senile cataractous lenses suggest that the ratio of AR to PD in human lenses is the reverse of the animal lens situation. The regulatory properties and cofactor requirements for human lens AR and PD have not been determined.

The main objective of this study is to evaluate the sorbitol pathway in human lenses on the enzymic level and to relate these findings to whole lens studies. For this purpose, we measured the activity of AR and PD on a per-lens and a specific-activity basis and compared them to animal lenses in which the sorbitol pathway is established to be operable. We correlated AR and PD levels in human lenses with type and age. In the case of AR we determined the substrate and cofactor requirements, i.e., the \( K_m \) values for glyceraldehyde, NADPH, and glucose. Enzyme activation by sulfate ions and inhibition by NADP are reported. In addition, the distribution of AR and PD in the lens epithelium, cortex, and nucleus was established. In the case of PD we partially purified the enzyme and determined the kinetic constants for sorbitol, xylitol, and NAD and NADH inhibition. \( K_m \) values for the reverse reaction were established for fructose, NADH, and NADPH. The pH optima for the forward and reverse reactions and heat stability were determined. Levels of NAD, NADH, NADP, and NADPH were measured in human lenses to ascertain the availability of nucleotides to AR and PD.

Methods and materials

Tissue preparation. Diabetic and nondiabetic human cataractous lenses were obtained from patients by intracapsular cryoprobe extraction at the New England Deaconess Hospital and the Massachusetts Eye & Ear Infirmary, respectively. Normal lenses were obtained from the New England Eye Bank within 24 hr after death. Lenses were then photographed and classified according
Table I. Comparison of AR and PD activities in various lens species*

<table>
<thead>
<tr>
<th>Lens species</th>
<th>AR</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (100 gm)</td>
<td>6</td>
<td>2.12 ± 0.30</td>
</tr>
<tr>
<td>Rat (1 kg)</td>
<td>2</td>
<td>5.88 ± 0.06</td>
</tr>
<tr>
<td>Rabbit (1.5 kg)</td>
<td>6</td>
<td>6.74 ± 0.38</td>
</tr>
<tr>
<td>Calf</td>
<td>2</td>
<td>2.78 ± 0.60</td>
</tr>
<tr>
<td>Human:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (52-83 yr)</td>
<td>6</td>
<td>0.8 ± 0.13</td>
</tr>
<tr>
<td>Diabetic cataract (54-75 yr)</td>
<td>4</td>
<td>0.43 ± 0.21</td>
</tr>
<tr>
<td>Nondiabetic cataract (52-94 yr)</td>
<td>28</td>
<td>28</td>
</tr>
</tbody>
</table>

*Activity (Δ O.D./5') is expressed per lens and per milligram of protein. (Sp. act.; ± S.D.; n is the number of lenses studied.

Enzyme, product, and protein determinations. AR was assayed by the method of Hayman et al.20 All assays were performed in the presence of 0.4M lithium sulfate unless specified otherwise. Controls for enzyme, substrate, and co-factor were run simultaneously with the complete reaction mixture. The high NADPH oxidation with supernatant alone and the moderate increase in 340 nm absorption with glyceraldelyde and supernatant above were subtracted from and added to the change in 340 nm absorptivity of the complete system. PD activity in the forward and reverse directions were assayed by the method of Jedziniak et al.4 at saturating levels of substrate and co-factor. Various aliquots of PD were assayed to ensure linearity of the reaction at levels low enough to obviate NADH inhibition. Glucose and fructose measurements in whole lenses were done according to the method of Bergmeyer.21 Sorbitol was determined according to the method of Sherman et al.22 Protein determinations were performed according to the method of Lowry et al.24 Purification of PD. Increments of saturated ammonium sulfate were added to the supernatant fraction to achieve 0 to 20%, 20% to 40%, and 40% to 60% saturation. All additions were done with gentle stirring at 4° C. The resultant precipitate was obtained by centrifugation at 20,000 × g for 10 min at 4° C. The first 40% to 60% precipitate was suspended in 0.065M phosphate buffer, pH 6.8, and centrifuged as described above. Enzyme, product, and protein determinations. AR was assayed by the method of Hayman et al.20 All assays were performed in the presence of 0.4M lithium sulfate unless specified otherwise. Controls for enzyme, substrate, and co-factor were run simultaneously with the complete reaction mixture. The high NADPH oxidation with supernatant alone and the moderate increase in 340 nm absorption with glyceraldelyde and supernatant above were subtracted from and added to the change in 340 nm absorptivity of the complete system. PD activity in the forward and reverse directions were assayed by the method of Jedziniak et al.4 at saturating levels of substrate and co-factor. Various aliquots of PD were assayed to ensure linearity of the reaction at levels low enough to obviate NADH inhibition. Glucose and fructose measurements in whole lenses were done according to the method of Bergmeyer.21 Sorbitol was determined according to the method of Sherman et al.22 Protein determinations were performed according to the method of Lowry et al.24 Purification of PD. Increments of saturated ammonium sulfate were added to the supernatant fraction to achieve 0 to 20%, 20% to 40%, and 40% to 60% saturation. All additions were done with gentle stirring at 4° C. The resultant precipitate was obtained by centrifugation at 20,000 × g for 10 min at 4° C. The first 40% to 60% precipitate was suspended in 0.065M phosphate buffer, pH 6.8, and enzyme-enriched protein was repurified in a similar step-wise fashion. The 40% to 60% ammonium sulfate suspension was eluted either in two stages from a SE G-100 column followed by SE S-200 column, or by ascending/descending chromatography on SE S-200 columns. In the former case, the columns measured 40 by 2.7 cm, and in the latter case a 55 by 2.6 cm ascending column was coupled to a 30 by 2.6 cm descending column. In each case, the Sephadex gel was equilibrated with 0.04M PO₄, pH 6.8, containing 0.2% sodium azide and 3 mM β-mEtoH. Absorptivity of 280 nm was monitored and related to enzyme activity. Nucleotide levels. Human cataracts were dissected into epithelial—superficial cortical and deep cortical fractions immediately after the cataract was extracted from the eye and quickly frozen with liquid N₂. These fractions were then homogenized.
in ice-cold water, divided into two aliquots, and rapidly mixed with equal volumes of either 0.4M KOH (for determination of NADH or NADPH) or 0.46M KH₂PO₄ (for determination of NAD or NADP). The mixtures were heated for 1 min in a boiling water bath, cooled on ice, neutralized with either 0.23M KH₂PO₄ or 0.2M KOH, and centrifuged at 12,800 x g for 2 min. In the supernatants, the co-factors were assayed according to the procedures of Allig et al.²⁵ with the following modifications. Each supernatant was assayed with two media containing an added internal standard or none, and the background was assayed with a medium containing no auxiliary enzyme. Recovery of the internal standards was generally more than 90% when compared with standards processed identically to the lens homogenates.

**Results**

**AR and PD in human and animal lenses.**

Table I compares the activity of AR and PD in adult human lenses to AR and PD levels in a variety of animal lenses. Human lenses were classified as normal (i.e., clear) and as having diabetic and nondiabetic cataracts. By a comparison of the AR and PD activities of all human lenses studied with those of the animal lenses, two important features emerged from the data. First, the level of AR in human lenses was roughly 1 order of magnitude lower than was found for the animal lens on a per lens basis. On a specific activity basis, human lens AR was also lower than all animal species with the exception of the calf.
By a comparison of the activities of AR and PD in the adult human lens situation, it can further be seen from Table I that the diabetic cataractous human lens was not distinguished from the nondiabetic cataract by having significantly higher levels of AR. This is the reverse of the observation by Chylack for incubated human lenses. Quite in contrast to studies with nondiabetic and diabetic rat lenses, the data in Table I show that, on a per lens basis, both a limited number of diabetic cataracts and a significant number of human nondiabetic cataracts contained a lesser capacity to produce sorbitol than did three normal lens pairs of the same age group. This difference was not reflected in a comparison of specific activities. Although sufficient numbers of diabetic cataracts and normal lenses were not available to permit a statistical analysis of the reliability of the values, no suggestion that the diabetic lens is characterized by elevated levels of AR was found.

**AR and PD as a function of cataract type.**

AR activity was measured in 27 nondiabetic cataracts, and PD activity was measured in 13. Cataracts were classified according to the severity of opacification with the Cooperative Cataract Research Group, and the volume of each cortical quadrant was estimated with elliptical geometry. The total and specific activities of AR and PD (forward and reverse reactions) were correlated with the increasing volume of cortical opacification per lens. It can be seen from Fig. 1, A, in the case of AR that a number of cataracts, regardless of the degree of cortical involvement, contained the same level of enzyme. If one considers the averages of the extremes of the data, there was a slight decrease in AR activity with increasing opacification. In the case of PD, Fig. 1, B, the specific activity was more clearly associated with the extent of cortical involvement. With the exception of one lens, all the data points show that cortical cataracts with 80% to 100% opacification contained roughly one-third the activity of PD relative to cataracts with no cortical involvement. The same pattern evolved on a per-lens basis. The figures suggest that a slight decrease in AR...
activity and a more drastic decrease of PD activity resulted from increased cataractogenesis in the lens cortex.

**AR and PD as a function of age.** Insight into the deleterious role that AR may play in the formation of human diabetic cataract arises not from comparing the activity of AR in senile human lenses of differing type (Table I) but rather from studying the levels of AR in all lens types as a function of age.

Fig. 2, A and B, summarizes the activity of AR measured on a per-lens and specific-activity basis, respectively, in normal, non-diabetic, and diabetic cataractous lenses ranging in age between 8 and 94 years. Although the degree of opacification of the lens undoubtedly influenced the activity of AR in cataractous lenses of all ages (cf. AR and PD as a function of cataract type), it clearly emerged from both figures that juvenile normal human lenses contained substantially higher levels of AR than did senile lenses of all types. Thus the juvenile lens, if exposed to high glucose levels, has the capacity to form two to three times the level of sorbitol produced by adult lenses. It is interesting to note in this context that the young human lens contained levels of AR comparable to those measured in the calf lens in terms of specific activity (Table I). On a per-lens basis, however, the young human lens remained lower in AR activity than any animal lens.

Figs. 3, A and B, and 4 demonstrate that in contrast to AR, PD levels on a per-lens and specific-activity basis in human in both the sorbitol-to-fructose and fructose-to-sorbitol directions remained fairly constant and persistently high with age.

**K<sub>m</sub> values for AR.** The substrate and cofactor requirements of adult human lens AR for glyceraldehyde and NADPH were measured. The S vs. V graphs for both determinations showed that AR followed regular Michaelis-Menten kinetics. The K<sub>m</sub> established for glyceraldehyde as substrate was 2.4 × 10⁻⁵M. An equivalent amount of cofactor, i.e., 6 × 10⁻⁵M NADPH, was required to produce one-half the maximum velocity (~0.4 O.D./5' /ml) of adult human lens AR. Although AR operated with glucose at a maximum velocity equal to that with glyceraldehyde as substrate, the concentration of glucose necessary to yield one-half the maximal velocity was considerably higher than with glyceraldehyde as substrate. The K<sub>m</sub> of glucose for human len AR was roughly 200 mM. Thus AR has roughly a 1000 times greater affinity for glyceraldehyde than for glucose as substrate.

**Activation and inhibition of AR.** Similar to the animal lens, human lens AR was both activated by Li₂SO₄ and inhibited by NADP, a reaction product. AR in young and old normal and diabetic and nondiabetic cataractous lenses was stimulated threefold to fourfold by 0.4M Li₂SO₄. Fig. 5, a plot of AR activity with and without NADP as a function of age.
NADPH concentration, reveals that AR was inhibited noncompetitively by NADP. The $K_i$ determined for this inhibition was $6.88 \times 10^{-5}$M. This $K_i$ was within the same order of magnitude determined for the $K_m$ of NADPH.

**Localization of AR and PD.** Table II shows that although human lens AR is an enzyme of low activity, its activity is almost exclusively confined to the lens epithelium. Roughly 70% of the capacity of the human lens to produce sorbitol is located in as little as 2% of the lens wet weight. This compartmentalization would produce a concentration of sorbitol 50 times greater than if AR were distributed evenly throughout the lens. In the case of the adult lens, with sufficient glucose as substrate, the $V_{max}$ of AR was approximately 0.4 O.D./5'/ml. By correcting this value for time, dilution, and sulfate stimulation, one can calculate that the adult human lens is capable of producing 6 to 7 $\mu$mol of sorbitol in 24 hr. Since 70% of human lens is confined to one-fiftieth the lens wet weight, the osmotic force exerted by sorbitol in the lens epithelium would be expected to be 35 times greater than on a per-lens basis. If saturating levels of glucose were available to the lens, it could be anticipated that sorbitol in the adult lens epithelium would exert an osmotic stress of some 190 mOsm. In the juvenile lens, which contains two to three times the level of AR present in the adult lens, as much as 380 to 570 mOsm sorbitol would be added to the osmolality of the lens epithelium. These osmolarities in both young and old human lenses are sufficient to lead to a hypertonic situation.

PD activity as is shown in Table II was more evenly distributed throughout the lens than AR. Of the total activity, 50% of PD was located in the lens epithelium and 40% in the cortex.

**Purification and molecular weight determination of PD.** Due to the abundance and marked stability of PD, 40% to 60% of a 60-to 100-fold pure enzyme preparation can be re-
covered following ammonium sulfate precipitation and chromatographic separation on Sephadex G-100 and Sephacryl S-200 superfine gels. Table III summarizes the protocol developed to obtain 100-fold pure PD from normal and diabetic and nondiabetic cataractous lenses. No differences in purification were observed dependent upon the source of enzyme. PD from the human lens elutes in the beta-crystallin region, that is, intermediate-molecular-weight range, of the lens crystallins. The molecular weight of PD measured from a calibration curve relating the elution ratio of ribonuclease, chymotrypsinogen, hemoglobin, and aldolase relative to blue dextran to the logarithm of their molecular weights was estimated to be 102,000 daltons.

**Kinetic properties**

Sorbitol to fructose. Table IV summarizes the K_m values for sorbitol, xylitol, and NAD and the K_i value for NADH determined for PD purified from senile normal and nondiabetic and diabetic cataracts. The velocity vs. substrate and co-factor concentration curves followed regular Michaelis-Menten kinetics in all cases. The values for K_m of sorbitol, xylitol, and NAD were determined from the intersection of the abscissa of a double reciprocal plot of 1/V vs. 1/S. The K_i value for NADH was determined by plotting 1/V vs. either inhibitor concentration or NAD concentration at different levels of NADH. The K_m of sorbitol for PD from all lens types was approximately the same, roughly 1.4 mM. Although PD from adult human lenses elicited approximately the same velocity with xylitol, sorbitol, and mannitol as substrate, the K_m for xylitol on the average was one-fourth that required to achieve maximum velocity with sorbitol. Full enzyme activity with mannitol as substrate distinguishes PD in human lenses from the sheep liver enzyme. Similar to the calf and sheep lens enzyme, human lens PD was found to be inactive with inositol, dulcitol, and glycerol in the forward direction. Identical to the reactivity of PD with substrate, no difference in the K_m value for co-factor was found to distinguish the enzyme of diabetic lenses from normal lenses and nondiabetic cataracts of the same age. The K_m for NAD in all situations was in the order of 5 × 10^-5M, a value one-fourth that required for substrate.

The K_i value calculated for NADH, 0.002 mM, was extremely low regardless of lens type. The competitive effect of NADH in inhibiting PD is shown in Fig. 6. The data demonstrate that NADH may represent a sensitive control mechanism for regulating the activity of PD in normal and diabetic and nondiabetic cataractous lenses.

Fructose to sorbitol. Fructose (40 mM) was required to reverse the activity of PD to the fructose-to-sorbitol direction. The reduction of fructose proceeded maximally at similarly low concentrations of co-factor as are found.

### Table III. Purification protocol for PD

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total units</th>
<th>Total protein</th>
<th>Sp. act.</th>
<th>% recovery</th>
<th>Fold purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>229</td>
<td>200</td>
<td>1.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2 × (NH_4)_2SO_4 precipitate (40-60%)</td>
<td>171.75</td>
<td>18.75</td>
<td>9.16</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>SE G-100 + SE S-200 gel</td>
<td>91.6</td>
<td>0.9</td>
<td>120</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>SE S-200 gel</td>
<td>137.4</td>
<td>1.14</td>
<td>120</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table IV. Kinetic properties of human lens PD*

<table>
<thead>
<tr>
<th>Lens type</th>
<th>K_m (mM)</th>
<th>K_i of NADH (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitol</td>
<td>1.450</td>
<td>0.180</td>
</tr>
<tr>
<td>Xylitol</td>
<td>0.333</td>
<td>0.333</td>
</tr>
<tr>
<td>NAD</td>
<td>0.050</td>
<td>0.333</td>
</tr>
</tbody>
</table>

*Enzymes were obtained from pooled human senile lenses. Values shown were determined from Lineweaver-Burk plots (for K_i) and 1/V vs. (1) plots (for K_m).
for the forward reaction. Only ~0.02 mM NADH was required to lead to the formation of sorbitol at one-half the maximal velocity of PD. Interestingly, although NADP did not substitute as a co-factor for NAD in the oxidation of sorbitol, NADPH reacted with PD to reduce fructose at 25% the maximal velocity with NADH. The $K_m$ for NADPH for PD was $2.7 \times 10^{-4}$ M. In view of the remarkably high activity of PD in human lenses (cf. Table I) and the low as well as nonspecific co-factor requirement of the enzyme, it appeared that given adequate fructose as substrate, PD has the potential to produce substantial sorbitol.

**Effect of AR inhibitor on PD.** Alrestatin, an inhibitor presumably selective for AR, inhibits sorbitol accumulation in intact lenses incubated at high glucose concentration. Due to the capacity of PD to lead to sorbitol production, the effect of Alrestatin on PD was evaluated. Quite intriguingly, alrestatin, at a concentration range of $10^{-4}$ M, inhibited PD in both the sorbitol-to-fructose and the fructose-to-sorbitol directions by 50%. Although the concentration of alrestatin that inhibited PD was 100 times that for AR, these findings show that alrestatin was not entirely specific for AR. At a high concentration, alrestatin can alter the activities of both enzymes of the sorbitol pathway of the human lens.

**pH optima and heat stability of PD.** The pH optima for the forward and reverse directions of PD were determined. Although the...
forward reaction with sorbitol as substrate showed a broad peak between pH 8.0 and 9.5, substantial activity also occurred at physiologic pH, pH 7.4. PD in the fructose-to-sorbitol direction demonstrated a sharp peak at pH 6.8, the same optimal pH established for AR. PD was stable over a temperature range of 0 to 40° C. Thus its activity was preserved at 37° C, the physiologic temperature used for most incubation protocols.

**Nucleotide levels.** After the co-factor and inhibitor requirements for AR and PD had been established, it was essential to determine the levels of oxidized and reduced nucleotides available to both enzymes in the intact human lens. Table V summarizes the amounts and concentration of triphosphopyridine and diphosphopyridine nucleotides in the epithelium and superficial cortex as well as in the deep cortex of senile cataractous human lenses. In the case of AR, 0.27 nmol, i.e., 0.02 mM, NADPH occurred in the epithelium and superficial cortex, the site of AR activity. This concentration of NADPH was one-third the level of NADPH required for the full activity of AR (0.06 mM). Equivalent concentrations of NADP (0.27 nmol; i.e., 0.02 mM) were present in the epithelium and superficial cortex and were roughly one-third the concentration of NADPH required to inhibit AR (0.07 mM). In the case of PD, NAD and NADH levels in the epithelium and su-
perficial cortex as well as in the deep cortex were sufficiently high for PD to be fully active in both the forward and reverse directions (0.6 and 0.2 mM, respectively). A concentration of NADH adequate to inactivate PD in the oxidation of sorbitol to fructose occurred in the epithelium (0.02 mM) as well as in the deep cortex (0.05 mM).

Discussion

Our experimental results lead to a number of conclusions regarding the enzymic properties of human lens AR and PD. A somewhat elusive quality remains, however, as to the activity of these enzymes in the intact lens.

These enzymic studies show that although, indeed, the adult human lens contains AR, the activity of AR on a per-lens and a specific-activity basis is limited compared to a variety of animal lenses (cf. Table I). Under optimal conditions, AR is capable of producing, at the most, 6 to 7 \( \mu \)mol of sorbitol per lens per 24 hr. The capacity for AR to produce sorbitol from 35.5 mM glucose on an enzymic basis is 0.8 \( \mu \)mol/lens/24 hr. This net capacity for sorbitol production on the enzymic level is entirely consistent with the polyol production observed by Chylack et al. \(^{15}\) and Varma \(^{14}\) upon incubation of adult human lenses in 35.5 mM glucose. It is likewise comparable to the level of polyol observed by Pirie and van Heyningen\(^9\) for a variety of freshly excised cataractous lenses from diabetic patients with moderately elevated blood sugar levels (250 mg/100 ml). Since the enzyme level of AR in diabetic lenses is not elevated relative to the AR level in nondiabetic human lenses, the activity per se of AR cannot account for more net polyol formation in diabetic vs. nondiabetic adult lenses.

The rather strict compartmentalization of AR to the epithelium in all lenses and the increased capacity of the juvenile lens to form sorbitol by AR offer the greatest insight into the role of AR in diabetic cataract formation thus far. The AR level present in the juvenile lens epithelium is capable of contributing an osmotic stress of some 380 to 570 mOsm sorbitol per epithelium per 24 hr. This osmolarity is of a magnitude known to lead to experimental cataract formation in intact rabbit lenses.\(^8\) If young lenses were exposed to sufficiently elevated levels of glucose, it would appear that the sorbitol pathway could cause the early chronological onset of diabetic cataract.

An elusive quality of the sorbitol pathway in the adult intact human lens arises from a consideration of the regulatory properties of AR. A schematic diagram of the sorbitol pathway and a summary of the enzymic requirements (Table VI) for adult lens AR and PD is shown below. The actual concentrations of substrates, co-factors, and inhibitors and their extremes of range measured in the intact human lens, diabetic and nondiabetic, are included. It is quite clear from the diagram that the high \( K_m \) for glucose, roughly 200 mM, is not met by the concentration of glucose present in either the intact diabetic (3 to 4.5 mM) or nondiabetic (0.7 to 2.2 mM) human lens. The low level of available co-factor NADPH (0.02 mM) relative to the \( K_m \) of NADPH (0.06 mM) and the borderline level (0.02 mM) of NADP, the noncompetitive inhibitor for AR, discourage the concept that AR in situ can operate at maximal velocity. Key to the fact that sorbitol does, in fact, accumulate in adult lenses freshly excised from diabetic patients with extremely high blood glucose levels\(^9\),\(^{14}\) may be Cheng's\(^{27}\) observation that HK is significantly decreased with age in the human lens. A higher ratio of AR to HK and a highly elevated glucose stress in the diabetic situation in all probability account for increased polyol production in adult human diabetic cataractous lenses.

These enzymic studies also show that in the case of PD, the human lens has a uniquely high capacity to oxidize sorbitol to fructose and to reduce fructose to sorbitol. The \( V_{\text{max}} \) for PD in the fructose-to-sorbitol direction is 10 times the rate in the sorbitol-to-fructose direction. The high level of PD relative to AR in human lenses may account for the observation that considerable polyol is in the fructose form in the human lens.
contrast, by virtue of low PD level, sorbitol is the predominant product of the sorbitol pathway in the animal lens. Assuming that fructose neither diffuses freely from the lens nor is metabolized to a great extent, the resultant fructose concentration in the human lens would serve as an additive osmotic stress to the lens equivalent to that exerted by sorbitol. Human lens PD remains persistently high with age and is distributed evenly in the epithelium and cortex. The decline in PD activity in diabetic and nondiabetic cataracts with increased severity of cortical cataract is consistent with the localization of 40% the activity of PD in the lens cortex.

It can clearly be seen in the schematic diagram that diabetic as well as nondiabetic adult human lenses contain in situ levels of substrate and co-factor satisfactory for maximal PD activity. The very low $K_m$ of PD for sorbitol (1.45 mM) and for NAD (0.06 mM) are met and favor a rapid oxidation of sorbitol to fructose. Although a sufficient level of NADH occurs to inhibit PD in situ, this does not occur, since fructose is elevated to the same degree as sorbitol in both the diabetic and nondiabetic lenses.

The equilibrium of the sorbitol-to-fructose and fructose-to-sorbitol reactions of PD may provide, in itself, an intriguing control mechanism for the sorbitol pathway in the human lens. Conceivably, if enough NADH were generated via the forward reaction, it may, at some point, inhibit PD. Reconversion of fructose to sorbitol with the accumulated reduced co-factor may then occur and account for the fact that sorbitol and fructose are in equivalent concentrations in the human lens.

Further work is in progress in our laboratory to elucidate fluxes through the sorbitol pathway in situ and to study the possible coupled production of sorbitol by AR and PD in the human lens.

References

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