Glycosidases of the crystalline lens
II. Alterations in diabetic cataracts

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Six glycosidases were determined in: (1) lenses of alloxan-diabetic rats; (2) rabbit lenses incubated in high-glucose media in vitro; and (3) human senile cataracts, diabetic senile cataracts, and autopsy lenses. In diabetic rat lenses, the activities of β-glucosaminidase, β-galactosidase, and β-fucosidase increased with cataract progression as compared to control lenses. Glycosidase activities, however, did not differ in human diabetic cataracts (patients with normal blood glucose levels) as compared to human senile cataracts or autopsy lenses. Incubation of rabbit lenses in high-glucose Tyrode's media did not affect the glycosidase activities as compared to rabbit lenses incubated in normal Tyrode's media.

Key words: Glycosidases, crystalline lens, diabetic cataracts, β-glucosaminidase, β-galactosidase, β-fucosidase, α-mannosidase, β-glucosidase, β-galactosaminidase

The thickening of basement membranes in small blood vessels of the kidney and retina1 and in epithelium of the ciliary body2 and the deposition of periodic acid-Schiff (PAS)-positive material in retinal exudates3 and in nodular Kimmelstiel-Wilson renal lesions4 represent abnormalities of glycoprotein metabolism associated with diabetes mellitus. Though the composition of carbohydrates and amino acids in diabetic glomerular basement membranes was shown to be similar to that of nondiabetic membranes, a decreased turnover of basement membranes5 and an increase in number and mobility of glycoprotein electrophoretic bands in human diabetic kidneys6 indicate probable molecular or structural glycoprotein alterations in diabetes mellitus. Whether this abnormality is due to increased synthesis, decreased breakdown, or synthesis of molecularly deranged glycoproteins which cannot be degraded by glycosidases has not yet been clarified.

In human and experimental diabetic cataracts, pathological changes occur in the membranes of lens fibers7 and in the lens capsule,8,9 which is chemically, structurally,10 and immunologically11 similar to other basement membranes. The higher incidence of extracapsular senile cataract extractions in human diabetic subjects12 and the decrease in soluble proteins in diabetic senile cataracts as compared to nondiabetic senile cataracts13 may indicate a greater fragility and abnormal permeability of the lens capsule in diabetes. In a previous study,14 lens glycosidases were described. Here, glyco-
sidase activities in human diabetic senile cataracts and in the lens and kidney of alloxan-diabetic rats are reported.

Materials and methods

**Enzyme assay.** The fluorometric determinations of β-galactosidase, β-glucosidase, α-mannosidase, β-glucosaminidase, β-galactosaminidase, and β-fucosidase were as described previously.¹⁴

**Lens incubation in vitro.** The preparation of normal (5.5 mmolar glucose) Tyrode’s solution and high-glucose (33 mmolar glucose) Tyrode’s solution and the incubation procedure were as previously described.¹¹

**Glycosidase activities in human cataracts.** Human autopsy eyes from the Illinois Eye Bank, Chicago, Illinois, were obtained within 24 hours after death. The globes were opened via an equatorial incision, and the lenses were removed, weighed, and stored in 1.0 ml. of H₂O at -70°F. until processed.

Human senile cataracts and diabetic senile cataracts were transferred immediately to the laboratory from the operating room of the University of Illinois Eye and Ear Infirmary. The lenses were weighed, photographed, and stored in 1.0 ml. of H₂O at -70°F. until processed. Preoperative fasting blood sugar levels and the duration of diabetes mellitus were obtained from the hospital records.

**Human lenses were homogenized in 2.0 ml. of distilled water in a mechanically driven, all-glass homogenizer at 2,000 r.p.m. for approximately ten seconds.**

**Glycosidase activities in alloxan-diabetic rats.** Forty 200 gram male albino rats, obtained from the Hormone Assay Laboratory, Chicago, Illinois, received intraperitoneal injections of alloxan monohydrate (175 mg. per kilogram), and those rats without glycosuria after another two days were eliminated from the study. Cataractous changes in diabetic rats examined under the slit lamp and being similar to those found in galactose-fed rats were classified according to their progression in five stages. The morphologic features of each diabetic cataract stage were:

- **Stage 1—complete ring of equatorial vacuoles;**
- **Stage 2—anterior extension of vacuoles and opaque plaques;**
- **Stage 3—anterior cortical vacuoles and posterior subcapsular opacity;**
- **Stage 4—clearing of cortex and nuclear cataract;** and
- **Stage 5—increased density of nuclear cataract and renewed cortical opacification (mature lens).**

These cataracts were killed with ether, their eyes were dissected, and the lenses were removed and carefully separated from adhering ciliary bodies. The lenses were weighed, stored in 0.5 ml. of H₂O at -70°F., and subsequently homogenized in 1.0 ml. of distilled water. Enzyme activities were determined at the optimal pH for each enzyme, as pH curves were found to be similar for normal and diabetic lenses.

Yield from each rat were removed after death, and wedge-shaped sections through the central areas were cut and the upper and lower poles discarded. The kidneys were combined, weighed, and stored at -70°F. in 1.0 ml. of H₂O. Subsequently, homogenization in 50 ml of distilled water was performed as described above.

**Results**

**Lens incubation in vitro.** Glycosidase activities did not differ between rabbit lenses incubated in 5.5 mmolar glucose Tyrode’s solution and 33 mmolar glucose Tyrode’s solution after 24 or 70 hours (Fig. 1).

**Glycosidase activities in human cataracts.** Table I shows the glycosidase activities in human autopsy (optically clear) and human cataractous lenses. Enzyme activities in 17 autopsy lenses, 18 senile cataracts, and 13 diabetic senile cataracts (in micro-

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Table I. Activities of glycosidases in human autopsy lenses, senile cataracts, and diabetic senile cataracts (micromoles per gram per hour)

<table>
<thead>
<tr>
<th>Glycosidase</th>
<th>Autopsy lenses (No. = 17)</th>
<th>Senile cataracts (No. = 18)</th>
<th>Diabetic senile cataracts (No. = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase</td>
<td>0.044 ± 0.014</td>
<td>0.043 ± 0.017</td>
<td>0.046 ± 0.012</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>0.047 ± 0.019</td>
<td>0.042 ± 0.014</td>
<td>0.049 ± 0.007</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>0.508 ± 0.309</td>
<td>0.343 ± 0.194</td>
<td>0.504 ± 0.212</td>
</tr>
<tr>
<td>β-Glucosaminidase</td>
<td>0.987 ± 0.485</td>
<td>1.135 ± 0.522</td>
<td>1.277 ± 0.618</td>
</tr>
<tr>
<td>β-Galactosaminidase</td>
<td>0.447 ± 0.131</td>
<td>0.454 ± 0.125</td>
<td>0.530 ± 0.081</td>
</tr>
<tr>
<td>β-Fucosidase</td>
<td>0.031 ± 0.017</td>
<td>0.027 ± 0.014</td>
<td>0.031 ± 0.015</td>
</tr>
</tbody>
</table>

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Glycosidase activities in diabetic cataracts in rats. Lens glycosidase activities in alloxan-diabetic rats were altered during the formation and progression of cataract (Fig. 2). \( \beta \)-Glucosaminidase activities increased approximately 300 per cent in lenses with Stage 1 cataracts, reached the higher levels in Stage 3 cataracts, and decreased in Stage 5 lenses to approximately 200 per cent of the control values. \( \beta \)-Galactosidase activities increased in each successive stage of cataract, rising above 200 per cent in Stage 5 cataracts. \( \alpha \)-Mannosidase activities increased to approximately 200 per cent in Stage 1 and then decreased with subsequent cataract progression. \( \beta \)-Fucosidase activities increased in Stages 3 and 5 but were not significantly different from
those in control lenses at other stages of cataract development. \(\beta\)-Glucosidase and \(\beta\)-galactosaminidase activities in cataractous lenses did not differ significantly from activities in control lenses.

**Glycosidase activities in kidneys of alloxan-diabetic rats.** Table II shows the glycosidase activities in kidneys of normal and alloxan-diabetic rats. Enzyme activities in normal and diabetic kidneys (in micromoles per gram per hour) were: \(\beta\)-galactosidase 48.1 ± 3.4 and 42.5 ± 4.8; \(\beta\)-glucosidase 24.5 ± 3.3 and 25.4 ± 4.6; \(\alpha\)-mannosidase 21.3 ± 2.3 and 29.1 ± 5.7; \(\beta\)-glucosaminidase 289 ± 38 and 302 ± 56; \(\beta\)-galactosaminidase 65.7 ± 12.6 and 69.5 ± 10.1; and \(\beta\)-fucosidase 26.8 ± 2.9 and 25.3 ± 3.7.

**Discussion**

Glycoproteins of the lens capsule and membranes of lens fibers have been previously assigned structural and cementing roles. The finding of lens glycosidases indicates catabolic pathways for glycoproteins and glycolipids are operative, and thus, a continuous turnover of lens glycoproteins is very likely. Glycosidase activation or inhibition may participate in more or less rapid degradation of lens fiber membranes during cataract formation.
Table II. Glycosidase activities in kidneys of normal and alloxan-diabetic rats (micromoles per gram per hour)

<table>
<thead>
<tr>
<th>Glycosidase</th>
<th>Normal kidneys (No. = 10)</th>
<th>Diabetic kidneys (No. = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase</td>
<td>48.1 ± 3.4</td>
<td>42.5 ± 4.8</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>24.5 ± 3.3</td>
<td>25.4 ± 4.6</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>21.3 ± 2.3</td>
<td>29.1 ± 5.7</td>
</tr>
<tr>
<td>β-Glucosaminidase</td>
<td>280 ± 38</td>
<td>302 ± 56</td>
</tr>
<tr>
<td>β-Calactosaminidase</td>
<td>65.7 ± 12.6</td>
<td>69.5 ± 10.1</td>
</tr>
<tr>
<td>β-Fucosidase</td>
<td>26.8 ± 2.9</td>
<td>25.3 ± 3.7</td>
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</table>

The initial biochemical alterations in human and animal lenses in diabetes mellitus are well documented. It is believed that elevated levels of glucose in the lens are metabolized to sorbitol and fructose, resulting in an osmotic inflow of water and vacuolation and hydropic swelling of lens fibers. The ensuing systematic destruction of the membranes of lens fibers leads to the large interfibrillar clefts filled with protein coagulum, described by Friedenwald and Rytel. Previously, we found lens glycosidases attached mainly to the lens capsule and membranes of lens fibers. It would therefore seem that, in diabetic lenses, the initial disruption of lens fiber membranes caused by osmotic hydration is associated with the liberation or activation of glycosidases which catalyze the systematic hydrolysis of the complex glycoproteins and glycolipids of the lens membranes. The increasing breakdown of the lens membranes throughout the progression of diabetic cataracts, the lack of prevacuolar glycosidase activation, and the progressive increase of glycosidase activities during cataract formation support this contention.

In contrast to the alloxan-diabetes experiments in rat lenses, human diabetic senile cataracts failed to show significant glycosidase increases as compared to autopsy lenses or human senile cataracts in nondiabetic persons. Only β-glucosaminidase appeared slightly increased in diabetic human cataracts, a finding in agreement with elevated levels of this enzyme found in the serum of diabetic patients. Whether this enzyme is activated by high glucose levels in tissues or plasma or whether it is linked to a biochemical defect unrelated to glucose metabolism is not known. However, prior to cataract surgery in the human beings of this study, hyperglycemia was meticulously controlled, a fact reflected in consistently normal preoperative blood sugar levels. Any alterations in lens glycosidase activities may have reverted to normal by the time of lens extraction and enzyme determinations.

The findings of normal glycosidase activities in kidneys of alloxan-diabetic rats would tend to indicate normal catabolic pathways for glycoproteins in this tissue in the animal model. However, alloxan-diabetic rats do not reveal glomerular deposition of PAS-positive material or abnormal accumulations of protein-bound carbohydrates as found in human Kimmelsteil-Wilson kidneys.

The complexity of basement membrane alterations and of glycoprotein metabolism in human and experimental diabetes mellitus is obvious. Hypotheses of an increased synthesis of normal and abnormal glycoproteins and a decreased breakdown of these substances have both been postulated but neither conclusively demonstrated. An increased rate of synthesis of glycoproteins was proposed by Spiro and Spiro, who found elevated glucosyltransferase and galactosyltransferase activities in alloxan-diabetic animals. Evidence indicating a decreased glycoprotein breakdown was offered by Lazarow and Spiedel, who found 1H-proline in the basement membranes of alloxan-diabetic rats was retained in excess of 90 days, while in normal rats it had disappeared between 30 and 60 days. Also, Schiller and Dorfman found that turnover rates of 14C- and 35S-labeled acid mucopolysaccharides in skin of diabetic rats were considerably slower than those in nondiabetic rats. It would appear, therefore, that in diabetes mellitus the tenuous equilibrium between the rate of synthesis and the rate of degradation of glycoproteins is upset. It is likely that the increased glycosidase levels of diabetic rat lenses are in-
sufficient to keep up with an elevated rate of glycoprotein synthesis. However, the correlation between activities of enzymes involved in the synthesis of complex glycoproteins and glycolipids, and the glycosidases must await further studies.

REFERENCES