Glycosidases of the crystalline lens

I. Effect of pH, inhibitors, and distribution in various areas of the lens and in subcellular fractions

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Six glycosidases, enzymes involved in glycoprotein-glycolipid breakdown, were determined in human, rabbit, and rat crystalline lens: β-galactosidase, β-glucosidase, α-mannosidase, β-glucosaminidase, β-galactosaminidase, and β-fucosidase. Peak activities of these enzymes were all at acidic pH values, but individual variations in pH optimum among enzymes and species were present. In the rabbit, glycosidase activities were 11 to 29 times higher in lens than in aqueous humor. In determinations in various areas of the lens, glycosidase activities were higher in the capsule-epithelium area than in the rest of the lens (on a wet weight basis). A variety of steroids inhibited β-glucosidase, whereas vitamin A palmitate stimulated β-glucosidase and β-fucosidase.

Key words: crystalline lens, β-galactosaminidase, β-glucosidase, α-mannosidase, β-glucosaminidase, β-galactosaminidase, β-fucosidase

In the crystalline lens, glycoproteins are found in the capsule,1 in the membranes and insoluble proteins of fibers and epithelial cells, and in the interfibrillar cement substance.2 Among the glycoproteins of the isolated lens capsule, Spiro and Fukushi1 found two carbohydrate-peptide units: one a glucose and galactose disaccharide linked by an O-glycosidic bond to hydroxylysine and the other a heteropolysaccharide compound of mannose, hexosamines, fucose, and sialic acid and joined to either serine or threonine.4 The glycoproteins of the lens fiber membranes, attached to insoluble lens protein, and sedimenting at 600 × g and 10,000 × g were identified by Dische5 as containing glucose, galactose, mannose, fucose, sialic acid, glucosamine, and galactosamine, though the specific carbohydrate-protein linkages were not determined.

Although fulfilling mainly structural and cementing roles, it is likely that turnover of lens glycoproteins does occur. Evidence supplied from studies in kidney indicates the synthesis of these compounds involves a series of glycosyl transferases which transfer activated sugars from nucleotides to specific protein acceptors on the membranes of endoplasmic reticulum.6 At this time, however, evidence for lens glyco-
protein catabolism via specific enzyme systems is lacking. The characteristics of lens glycosidases, involved in the breakdown of the carbohydrate portion of glycoproteins and glycolipids are reported here.

Materials and methods

Preparation of tissues. Albino rabbits, weighing three kilograms, from Scientific Small Animal Farms, Arlington Heights, Illinois, were killed by intracardiac air injections, and the eyes were immediately removed. After opening the globes at the posterior pole, the vitreous gels were removed and the lenses carefully separated with blunt scissors from their zonular attachments. The lenses were weighed and stored in 1.0 ml of distilled water at -70° F. until processed.

Albino rats, weighing 200 grams, from the Hormone Assay Laboratory, Chicago, Illinois, were killed by ether administration. The lenses were removed via a posterior incision, carefully separated from the adhering ciliary bodies, weighed, and stored in 0.5 ml of distilled water at -70° F. until processed.

Lenses from human eyes, obtained within 24 hours after death from the Illinois Eye Bank, Chicago, Illinois, were removed, weighed, and stored in 1.0 ml of distilled water at -70° F. until processed.

To lenses of the various species, water was added in the following volumes: rabbit, 2.5 ml.; rat, 1.0 ml.; and human, 2.0 ml.; the lenses ground by a motor-driven all-glass homogenizer at 2,000 r.p.m. for approximately ten seconds.

Assay of enzymes. One hundred microliters of 0.1M citrate phosphate buffer of optimal pH for each enzyme, 10 μl of lens homogenate (similar volumes were used for determinations in aqueous humor and serum), and 100 μl of the corresponding 4-methylumbelliferyl glycoside, 1 nmol (0.5 mM for N-acetyl-β-D-galactosaminide), were incubated in Pyrex fluorometer tubes 10 x 75 mm. in a 37° C. shaking water bath for one hour. The reaction was terminated with 1.0 ml of 1.0M glycine-NaOH-NaCl buffer, pH 10.3. The fluorescence of the liberated 4-methylumbelliferone was measured on a Turner Fluorometer Model No. 111 with a Wratten 760 (365 mλ) primary filter and a Wratten 48 and 2A (450 mλ) secondary filter. 4-Methylumbelliferone standards were used, and blanks contained lens homogenate and buffer with the corresponding substrate added after strong alkali inactivation. Enzyme activities were calculated in lens homogenates in micromoles per gram per hour and in lens subcellular fractions in micromoles per gram of protein per milliliter per hour.

Chemicals. 4-Methylumbelliferyl-β-D-galactopyranoside, 4-M-N-acetyl-β-glucosaminide, 4-M-N-A-β-D-galactosaminide, 4-M-β-D-fucopyranoside, and 4-methylumbelliferone were obtained from the Pierce Chemical Company, Rockford, Illinois.

H₂O-dispersible vitamin D₃, estradiol, diethylstilbestrol, testosterone, progesterone, prednisone, vitamin A acetate, vitamin A palmitate, cortisone acetate, heparin sodium, methyl testosteron, and chondroitin sulfate were obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio.

Digitonin, α-L-fucose, N-acetylglucosamine, N-acetylgalactosamine, urea, D-sorbitol, and β-fructose were obtained from Sigma Chemical Corporation, St. Louis, Missouri.

Dextrose and inositol were obtained from the Fisher Scientific Company, Fair Lawn, New Jersey.

Subcellular fractionation. Eight rabbit lenses were homogenized in 25 ml of distilled water. To one 2.5 ml aliquot, an equal volume of H₂O was added, while to another 2.5 ml aliquot, an equal volume of 0.5M sucrose was added (resulting in a 0.25M solution). The paired samples were centrifuged in a Lourdes Beta-Fuge Model A-2 (4° C.) at 600 x g for 15 minutes and then at 10,000 x g for 30 minutes. These fractions contained lens capsules, mitochondria, and insoluble proteins with lens fiber membranes (glycoproteins). After each centrifugation, the supernatants were decanted and the sediments suspended in 1.0 ml of distilled water. Protein determinations in all samples were performed according to Lowry and associates.

Lens incubations in vitro. Normal Tyrode’s solution was prepared containing (in millimoles per liter) NaCl, 137; KCl, 2.68; CaCl₂ • 2H₂O, 2.04; MgCl₂ • 6H₂O, 0.49; Na₂HPO₄, 0.416; NaHCO₃, 11.9; and D-glucose, 5.5 (100 mg. per cent). High-glucose Tyrode’s solution was prepared by adding D-glucose 33mM (600 mg. per cent) instead of 5.5mM. Assuming lens requirements of 3 mmoles glucose per kilogram per hour, the media contained glucose in slight excess of that necessary to maintain glycolysis for 24 hours. After being gassed with 95 per cent O₂ and five per cent CO₂, 20 ml of each Tyrode’s solution were added to Kjeldhal round-bottom flasks in a 37° C. shaking water bath. From each rabbit, one lens was incubated in normal Tyrode’s and the other lens in high-glucose Tyrode’s. After 24 and 48 hours, the lenses were removed from the culture medias, weighed, stored in 1.0 ml of water at -70° F., and subsequently homogenized in 2.5 ml of distilled water.
Inhibition of lens glycosidases. Chemicals tested for their effects on lens glycosidases were prepared in the following concentrations: H₂O-dispersible vitamin D₃, digitonin, α-L-fucose, N-acetyl-glucosamine, N-acetyl galactosamine, dextrose, urea, D-sorbitol, and β-fructose 5mM; estradiol, diethylstilbestrol, D-glucose, and inositol 4mM; testosterone 3.5mM; progesterone, vitamin A acetate, and prednisone 3mM; cortisone acetate 2.5mM; vitamin A palmitate 2mM; heparin sodium 0.6mM; methyl testosterone 0.4mM; and chondroitin sulfate 0.25 mM.

One hundred microliters of 0.1M citrate phosphate buffer of optimal pH for each enzyme, 10 μl of rabbit lens homogenate, and 100 μl of inhibitor were incubated in a 37°C shaking water bath for 30 minutes prior to the addition of the appropriate 4-methylumbelliferyl glycoside and completion of enzyme assay as previously described.

Inhibition or stimulation was expressed in percentage change of enzyme activity from that in lens homogenates without any additional chemicals.

Topical distribution of lens glycosidases. Four lenses from freshly killed rabbits were decapsulated by blunt dissection and then quickly frozen and divided into four sections containing the equatorial ring, anterior cortex, nucleus, and posterior cortex, respectively. The samples were weighed and homogenized in 0.5 ml of distilled water, and the glycosidase activities were determined.

Results

Effect of pH. pH distributions from 3.6 to 7.6 for human, rabbit, and rat lens glycosidases are shown in Figs. 1, 2, and 3. The peak activities of these enzymes were generally at acidic pH values. The pH optimums in human, rabbit, and rat lenses, respectively, were: β-galactosidase 4.9, 4.1, and 3.6; β-glucosidase 4.9, 5.6, and 4.9; α-mannosidase 7.3, 6.4, and 6.4; β-glucosaminidase 5.3, 5.3, and 4.5; β-galactosaminidase 3.6, 4.9, and 6.1; and β-fucosidase 6.8, 4.9, and 4.1. The pH distributions were similar for α-mannosidase and β-glucosaminidase in all species, for β-galactosaminidase in human and rabbit, for β-glucosidase in human and rat, and for β-galactosidase in rabbit and rat. In human lenses, β-galactosidase, β-glucosidase, β-galactosaminidase, and β-fucosidase activities were relatively independent of pH variations, i.e., less than 50 percent activity change in the measured range.
Similar pH-independent activities were found for rabbit lens β-glucosidase and β-galactosaminidase and for rat lens β-fucosidase and β-galactosaminidase. Glycosidase activities at pH optimum and at physiologic pH are shown in Table I and Fig. 4, respectively. At pH optimum, the glycosidase with the greatest activity...
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Fig. 4. Comparative glycosidase activities of human, rabbit and rat lenses at pH 7.3. Each bar represents mean of each group.

Table I. Activities of glycosidases in human, rabbit, and rat lenses at pH optimum (micromoles per gram per hour)

<table>
<thead>
<tr>
<th>Glycosidase</th>
<th>Mean ± S.D.</th>
<th>Human (No. = 12)</th>
<th>Rabbit (No. = 12)</th>
<th>Rat (No. = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase</td>
<td>0.044 ± 0.014</td>
<td>0.236 ± 0.052</td>
<td>0.437 ± 0.185</td>
<td></td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>0.045 ± 0.020</td>
<td>0.046 ± 0.017</td>
<td>0.152 ± 0.032</td>
<td></td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>0.525 ± 0.355</td>
<td>0.225 ± 0.065</td>
<td>0.554 ± 0.128</td>
<td></td>
</tr>
<tr>
<td>β-Glucosaminidase</td>
<td>1.024 ± 0.256</td>
<td>1.034 ± 0.212</td>
<td>3.403 ± 1.435</td>
<td></td>
</tr>
<tr>
<td>β-Galactosaminidase</td>
<td>0.481 ± 0.137</td>
<td>0.503 ± 0.158</td>
<td>2.187 ± 0.147</td>
<td></td>
</tr>
<tr>
<td>β-Fucosidase</td>
<td>0.026 ± 0.014</td>
<td>0.116 ± 0.104</td>
<td>0.076 ± 0.016</td>
<td></td>
</tr>
</tbody>
</table>

in human, rabbit, and rat lenses was β-glucosaminidase, measuring (in micromoles per gram per hour) 1.024, 1.034, and 3.403, respectively. The glycosidase with the least activity in human and rat lens was β-fucosidase, (0.026 and 0.076) and in rabbit lens was β-glucosidase (0.046). At pH 7.3, β-galactosidase, β-glucosidase, β-glucosaminidase, and β-galactosaminidase activities were highest in rat lens, while α-mannosidase was highest in human lens and β-fucosidase was highest in rabbit lens. At pH optimum, all lens glycosidase activities were highest in the rat lens with the exception of β-fucosidase which was highest in the rabbit.

Enzyme kinetics. Operational Km values in freshly homogenized rabbit and rat lenses calculated from Lineweaver-Burke plots were (in millimoles per liter): β-galactosidase 0.125 and 0.45, β-glucosidase 0.08 and 0.83, α-mannosidase 0.03 and 0.44, and β-glucosaminidase 0.5 and 0.33; and in rat lenses, β-galactosaminidase 0.5 and β-fucosidase 2.0. Vmax values in rabbit and rat lenses were (in micromoles per gram per hour): β-galactosidase 0.45 and 0.71, β-glucosidase 0.09 and 1.35, α-mannosidase 0.35 and 0.83, β-glucosaminidase 5.0 and 5.0; and in rat lenses, β-galactosaminidase 5.0 and β-fucosidase 1.0.

Glycosidases in lens, aqueous humor,
Rabbit glycosidase activities were much higher in the lens than in the aqueous humor at pH 4.4 (Table II). Lens–aqueous humor ratios were: β-galactosidase 26, β-glucosidase 15, α-mannosidase 19, β-glucosaminidase 15, β-galactosaminidase 11, and β-fucosidase 29.

With the exception of β-glucosidase, glycosidase activities were greater in serum than in aqueous humor. Aqueous humor:serum ratios were: β-galactosidase 0.17, β-glucosidase 1.5, α-mannosidase 0.03, β-glucosaminidase 0.07, β-galactosaminidase 0.33, and β-fucosidase 0.66.

Subcellular fractionation. The distribution of lens glycosidases in the lens capsule and membrane-containing fractions, the supernatants, and the total homogenates are shown in Fig. 5. There was no significant difference between glycosidase activities in fractions prepared in water and 0.25M sucrose. Maximal glycosidase activities in the 10,000 × g fraction (600 × g for β-glucosaminidase) were (in percentage of activity in the lens homogenate): β-galactosidase 525 per cent, β-glucosidase 465 per cent, α-mannosidase 235 per cent, β-glucosaminidase 35 per cent, β-galactosaminidase 810 per cent, and β-fucosidase 525 per cent.
activities in the total lens homogenates and in the supernatants of the 10,000 × g fraction did not differ significantly.

**Lens incubations in vitro.** Glycosidase activities in rabbit lenses cultured in vitro for 24 and 70 hours were increased as compared with freshly obtained lenses (Fig. 6). The increase was linear for 70 hours and statistically significant (p < 0.01) for β-galactosidase, β-glucosaminidase, and β-galactosaminidase. The increase was neither linear nor statistically significant for β-glucosidase, α-mannosidase, or β-fucosidase.
Fig. 7. Activation of $\beta$-glucosidase and $\beta$-fucosidase by vitamin A palmitate. Each bar represents mean of two separate experiments, including fourteen pooled lenses.

Table III. Effect of inhibitors on rabbit lens glycosidase activities

<table>
<thead>
<tr>
<th>Glycosidase</th>
<th>Inhibitor</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mM)</td>
<td>0.12</td>
</tr>
<tr>
<td>$\beta$-Glucosidase</td>
<td>Testosterone</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Methyl testosterone</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Estradiol</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Prednisone</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Digitonin</td>
<td>16</td>
</tr>
<tr>
<td>$\alpha$-Mannosidase</td>
<td>Diethylstilbestrol</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Vitamin A acetate</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Digitonin</td>
<td>21</td>
</tr>
<tr>
<td>$\beta$-Glucosaminidase</td>
<td>D-Glucuronic acid</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Heparin sodium</td>
<td>5</td>
</tr>
<tr>
<td>$\beta$-Fucosidase</td>
<td>Diethylstilbestrol</td>
<td>15</td>
</tr>
</tbody>
</table>

**Effect of inhibitors.** Table III shows the inhibition of lens glycosidases by various chemicals in concentrations (in the final reaction mixture) from 0.12 mmole per liter to 2.4 mmole per liter. Inhibition of $\beta$-glucosidase (inhibitor concentrations = 2.4 mmole per liter) were: testosterone 27 per cent, methyl testosterone 23 per cent, estradiol 35 per cent, progesterone 27 per cent, prednisone 29 per cent, and digitonin 26 per cent; of $\alpha$-mannosidase: diethylstilbestrol 25 per cent, vitamin A acetate 43 per cent, and digitonin 42 per cent; and of $\beta$-glucosaminidase: D-glucuronic acid 35 per cent and heparin sodium 18 per cent.
Table IV. Distribution of glycosidases in rabbit lenses (micromoles per gram per hour)

<table>
<thead>
<tr>
<th>Glycosidase</th>
<th>Capsule and epithelium</th>
<th>Equatorial ring</th>
<th>Anterior cortex</th>
<th>Nucleus</th>
<th>Posterior cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase</td>
<td>1.145 ± 0.583</td>
<td>0.148 ± 0.035</td>
<td>0.140 ± 0.028</td>
<td>0.123 ± 0.025</td>
<td>0.139 ± 0.005</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>0.033 ± 0.004</td>
<td>0.037 ± 0.017</td>
<td>0.023 ± 0.010</td>
<td>0.014 ± 0.004</td>
<td>0.024 ± 0.005</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>0.236 ± 0.176</td>
<td>0.421 ± 0.064</td>
<td>0.301 ± 0.106</td>
<td>0.396 ± 0.080</td>
<td>0.386 ± 0.077</td>
</tr>
<tr>
<td>β-Glucosaminidase</td>
<td>5.454 ± 2.730</td>
<td>2.717 ± 0.882</td>
<td>1.819 ± 0.724</td>
<td>0.916 ± 0.342</td>
<td>1.421 ± 0.567</td>
</tr>
<tr>
<td>β-Calactosaminidase</td>
<td>1.378 ± 0.533</td>
<td>0.582 ± 0.294</td>
<td>0.605 ± 0.168</td>
<td>0.275 ± 0.091</td>
<td>0.526 ± 0.164</td>
</tr>
<tr>
<td>β-Fucosidase</td>
<td>0.120 ± 0.048</td>
<td>0.033 ± 0.008</td>
<td>0.036 ± 0.002</td>
<td>0.026 ± 0.001</td>
<td>0.038 ± 0.001</td>
</tr>
</tbody>
</table>

Incubation with vitamin A palmitate resulted in stimulation of β-glucosidase and β-fucosidase (Fig. 7). When 10 mmolar concentrations of the chemical were used, activities rose to 250 and 270 per cent of control values, respectively. Incubation with vitamin A palmitate resulted in no significant alteration of β-galactosidase, α-mannosidase, β-glucosaminidase, and β-galactosaminidase.

Digitonin, 2.4mM, increased β-glucosaminidase activities 10 per cent, decreased β-glucosidase and α-mannosidase activities 26 per cent and 42 per cent, respectively, and had no effect on β-galactosidase, β-galactosaminidase, and β-fucosidase.

N-acetyl glucosamine (5mM per liter) and N-acetyl galactosamine (5mM per liter) inhibited β-glucosaminidase 27 per cent and β-galactosaminidase 25 per cent, respectively.

Triton-X-100 (1 per cent), H2O-dispersible vitamin D3, urea, s-sorbitol, β-fructose, chondroitin sulfate, hyaluronic acid, inositol, and cortisone acetate in the concentrations tested did not appreciably affect lens glycosidase activities.

Discussion

The low glycosidase activities in lenses of human beings, rabbits, and rats do not allow the application of spectrophotometric procedures or the use of lens or other biological glycoprotein substrates for their determination. The fluorometric procedures based on the degradation of 4-methylumbelliferyl glycosides are extremely sensitive and thus well suited for determinations of lens glycosidases. The tissue blanks are quite low due to the thousandfold dilution of the original tissue.

When compared to other tissues, glycosidase activities in the crystalline lens are extremely low. In rat tissues, β-glucosaminidase, β-galactosidase, and α-mannosidase activities (in micromoles per gram per hour) were, respectively: kidney 85, 76, 26; liver 39, 32, 12; epididymis 296, 326, 520; and pancreas 6, 8, 18; whereas in lens there were 3.4, 0.4, and 0.6. β-galactosidase and β-glucosidase in rat brain (in micromoles per gram per hour) were 13.7 and 1.4, while in the lens were 0.4 and 0.2. In human tissues, β-glucosaminidase, β-galactosidase, and α-mannosidase activities (in micromoles per gram per hour) were, respectively: kidney 95, 27, 25; and aorta 0.05, 0.04, 0.06; whereas in the human lens they were 1.0, 0.4, and 0.4.

The high glycosidase activities in the lens compared to those in its surrounding fluid, aqueous humor, indicate local syn-
thesis within the tissue. Since the molecular weight of glycosidases is between 50,000 and 200,000, it appears unlikely that active transport of enzymes into the lens could take place in vivo. Also, lenses incubated in vitro in Tyrode's media (no enzymes included) for 24 hours showed increased activities of five glycosidases.

In four lenses dissected into five sections—capsule and epithelium, equatorial ring, anterior cortex, posterior cortex, and nucleus—glycosidase activities, with the exception of α-mannosidase, were highest (on a wet weight basis) in the capsule and epithelium and lowest in the cortex and nucleus. However, most of the mass of the lens is composed of lens fibers, and total glycosidase activities were significantly higher in the cortex and nucleus where the fiber membranes are located. In the subcellular fractions, glycosidases were two- to sevenfold higher in the fractions containing fragments of lens capsule, membranes, heavy mitochondria, and insoluble proteins than in the supernatants. Spiro and Fukuski found a high concentration of glycoproteins in the lens capsule which is known to stain intensely with periodic acid-Schiff (PAS) and Dische found glycoproteins associated with lens fiber membranes. Thus, a close association of lens glycosidases with their substrates is evident. This relationship is even further strengthened as renal and hepatic glycosidases stain with PAS, indicating their glycoprotein nature.

The finding of glycosidase activities within the membrane fractions most likely indicates a turnover of such membranous material, a new concept considering the structural and antigenic roles assigned to them in the past. Unfortunately, the molecular weight and structural sequence of lens glycoproteins has not been determined, and, therefore, turnover rates from glycosidase activities cannot be calculated. It is likely, however, that the low-activity glycosidases of the lens (β-fucosidase and β-glucosidase) represent rate-limiting enzymes in the breakdown of lens glycoproteins and glycolipids.

It is generally believed, due to the results of studies on rat liver, spleen, muscle, and brain, and human kidney that the glycosidases are contained within lysosomes. Several investigators have shown that liver β-galactosidase, β-glucosidase, and β-glucosaminidase and brain β-glucosidase are associated with the lysosomal membrane rather than with the soluble portions of these particles. In the lens, however, lysosomes have never been identified by electron microscopy. Also, lens glycosidases sediment in the membrane rather than in the supernatant fraction, and detergents such as triton-X-100 and digitonin that release glycosidases from liver or brain lysosomes do not significantly activate lens glycosidases. Furthermore, whereas liver lysosomal activities are increased by incubation with digitonin, β-estradiol, testosterone, and progesterone and not affected by vitamin A palmitate, lens glycosidases are either not affected or inhibited (β-glucosidase) by digitonin and the steroid hormones and markedly increased by vitamin A palmitate.

Lysosomes represent membrane-bound particles containing inactive enzymes which when activated serve specific hydrolytic functions in complex cells such as those of secretory organs or CNS tissue. In the lens however, with a low metabolism and no secretory or highly specialized functions, it appears that evolutionary differentiation of membranes to form lysosomes has not taken place.

It is of interest that the low β-glucosidase activities, indicative of a rate-limiting role, are further decreased by steroid derivatives. Since in human beings systemic or topical steroid administration are known to induce cataracts and systemic deficiency of α-mannosidase has been associated with cataracts, it is possible that normal lens glycosidase function is necessary for optical transparency.

*Cohen, A. I.: Personal communication.
It is likely that several molecular forms of each glycosidase exist in the lens. However, the low enzymatic activities in lens homogenates did not allow glycosidase separation or even detection by starch gel electrophoresis. Investigations into the molecular structure of these enzymes must therefore await their further purification.

The authors wish to express their appreciation to Miss Arlene Duwelius for her technical assistance.

REFERENCES