Changes associated with the appearance of mature sugar cataracts

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Analyses for lens sugar alcohols, fructose, glucose, ATP, dry weight, hydration, and extracellular space were made before and after the appearance of mature diabetic and galactose cataracts. The appearance of mature cataracts in galactose-fed rats is associated with a marked and sudden decrease in the level of lens sugar alcohol, ATP, and dry weight, and an increase in lens hydration and extracellular space, as determined with radioactive sorbitol. Similarly, in the lenses from diabetic rats there is a sudden decrease in lens sugar alcohol, fructose, ATP, and dry weight, and an increase in lens glucose, hydration, and extracellular space. The results are interpreted as indicating that the appearance of mature cataracts is associated with the disruption of the fiber membranes of the lens.

In diabetic and galactose-fed rats the appearance of a mature lens cataract, as a white opacity which is visible to the naked eye, is sudden and accompanied by striking physiologic and biochemical changes.1 It is the purpose of this paper to present, in a graphic manner, the course of these changes during the cataractogenic period, plus additional new observations, so that trends and abrupt changes may be noted.

Methods

Mature cataracts were determined with the naked eye by observing the lens daily and noting the day on which the lens became opaque. In rats, this change is sudden and the day of onset of a mature cataract is readily determined.

Diabetes was produced in 27 to 38-day-old male Sprague-Dawley rats by the intravenous injection of 50 mg. of alloxan monohydrate per kilogram of body weight. Only rats with average blood sugars above 400 mg. per cent were used. The average blood sugar was about 500 mg. per cent and the median time for mature cataract formation was 76 days.

The galactose diet consisted of a mixture of 65 per cent ground chow and 35 per cent galactose. It was fed in unlimited amounts to male Sprague-Dawley rats having an initial age of 25 to 27 days. The median time for mature cataract formation was 18 days.

The lenses were removed as previously described.5 Individual lenses were macerated in 1 ml. of 2.0 per cent zinc sulfate and 1 ml. of 1.8 per cent barium hydroxide. The proteins were removed by centrifuging and 0.5 aliquots were used for determining reduced sugars, fructose, and glucose. Sorbitol and dulcitol were determined by the method of West and Rapoport3 as modified by Faulkner4; glucose by the oxidase method5; and fructose by the method of Roe.6 For the determination of ATP, individual lenses were macerated in 1 ml. of water; the mixture was heated for 5 minutes in boiling water, and the proteins were removed by centrifuging. A 0.2 ml. aliquot of the supernatant was used to determine ATP in a Farrand fluorometer with the firefly lantern method.7 Results are reported as milligrams of the disodium salt per 100 Gm. of lens. The
extracellular space was estimated by measuring the sorbitol space of individual lenses incubated with tritium-labeled sorbitol. Chemicals used as standards were obtained as follows: sorbitol, fructose, and glucose (Nutritional Biochemicals Corporation); ATP disodium salt (Sigma Chemical Company); and tritium-labeled sorbitol (New England Nuclear Corporation).

The dry weight was determined by drying the lens to constant weight in a 90° oven. The hydration index was determined by dividing the wet weight by the dry weight.

Results

The results obtained with diabetic and galactose-fed rats are shown in Figs. 1 and 2. Values are expressed on the basis of wet lens weight for sugar alcohols, fructose, glucose, ATP, and extracellular space. The left side of each figure shows the course during the period before the appearance of mature cataracts, and the right side shows the changes that are found at different times after the appearance of mature cataracts.

The normal level of sugar alcohol in the lenses of rats is low. Kinoshita, Merola, and Dikmak reported a value of 27 mg. per 100 Gm. Kuck studied the change with age in 97 lenses and found that the level increased from 17 to 34 mg. per 100 Gm. as the age changed from 21 days to 200 days. An analysis in this laboratory of the lenses of 5 rats at 200 days of age gave a value of 49 mg. per 100 Gm.

The normal level of lens fructose in rats also varies with age. Kuck reported a value of 27 mg. per 100 Gm. Kuck studied the change with age in 97 lenses and found that the level increased from 17 to 34 mg. per 100 Gm. as the age changed from 21 days to 200 days. An analysis in this laboratory of the lenses of 5 rats at 200 days of age gave a value of 49 mg. per 100 Gm.

The normal level of lens fructose in rats also varies with age. Kuck found that the level increased from 6.5 to 12 mg. per 100 Gm. as the age changed from 21 to 100 days, and from 15 to 27 mg. per 100 Gm. as the age advanced from 110 to 200 days. Lerman and Kinoshita, Merola, and Dikmak reported values for young rats of 8.1 to 11.2 and 14.6 mg. per 100 Gm., respectively. van Heyningen found levels of 139 to 183 mg. per 100 Gm. in 24 to 42-day-old rats.

The dry weight of lenses from normal rats increases linearly at a rate of 1.8 mg. per 10 days. The values for 47 rats between 25 and 55 days of age increased from 6.4 to 11.8 mg. At 55 days the dry weight represents 40.5 per cent of the wet weight. The protein content of the normal rat lens averages 37.8 per cent of the wet weight at 35 days, 38.8 per cent at 55 days, and 39.2 per cent at 75 days. Protein thus accounts for 96 per cent of the dry weight, and the dry weight may be interpreted as an indicator of the protein level.

The hydration index of the normal rat lens changes slightly with age. For three groups of rats ranging in age from 25 to 34, 35 to 44, and 45 to 55 days, the hydration index decreased from 2.61 to 2.53 to 2.47, respectively. This last value agrees with the finding of Salit, Swan, and Paul for 50 mg. (over 200 days old) rat lenses.

Sorbitol has been used to measure the extracellular space because it does not penetrate the cell membrane and because its characteristics are similar to those of glucose. Sorbitol was used to determine the extracellular space because it does not penetrate the cell membrane and because its characteristics are similar to those of glucose. The sorbitol space was determined on lenses from 12 normal rats ranging in age from 27 to 44 days. The values found were between 5 and 18 μl per 100 mg. with an average value of 9.9 μl. The sorbitol space may vary somewhat with the conditions of incubation. Another series of 100 lenses from approximately 30-day-old rats that was incubated in medium...
Fig. 1. The level of lens components before the appearance of mature cataracts in diabetic rats (blocks on the left side), and the levels after the appearance of mature cataracts (blocks on the right side). The straight lines were calculated by the method of least squares.
Fig. 2. The level of lens components before the appearance of mature cataracts in galactose-fed rats (blocks on left side), and the levels after the appearance of mature cataracts (blocks on right side). The straight line for extracellular space indicates the average value for all the determinations.
containing 1,000 mg. per cent glucose instead of usual 100 mg. per cent had a mean sorbitol space of 13.5 \mu l per 100 mg.

The changes observed in the period before the appearance of mature cataracts are in qualitative agreement with those of previous reports. Following the feeding of a diet containing a high concentration of galactose, the levels of dulcitol\(^6\)\(^{10}\) and hydration\(^6\) are increased in the lens, and the ATP level\(^17\) is decreased. The lenses of diabetic rats show similar changes with increases in the level of sorbitol\(^8\)\(^{10}\), glucose\(^18\) and fructose\(^18\) and decreases in the level of ATP.\(^10\)

Associated with the appearance of a mature cataract in galactose-fed and diabetic rats there are abrupt changes in all of the lens components that were measured. In galactose-fed rats the dulcitol, ATP, and dry weights decrease and the extracellular space and hydration index increase. In diabetic rats similar changes are observed. In addition, the lens glucose is increased and the lens fructose is decreased.

**Discussion**

Friedenwald\(^19\) has shown that the capsule of the lens is impermeable to large protein molecules, but freely permeable to smaller molecules. Becker\(^20\) has shown that the removal of the capsule with collagenase does not interfere with the functional integrity of the isolated lens and that cation transport proceeds in a normal manner. These observations, and others,\(^21\) demonstrate the fact that the fiber membrane of the lens is acting as a semipermeable cell membrane. With the appearance of mature cataracts, the dry weight drops to 47 per cent of its previous level. Since the dry weight reflects the protein level, this change indicates a sudden loss of lens proteins and confirms the observation of Dische.\(^12\) The sudden loss of proteins is best explained by concluding that fiber membranes are suddenly disrupted, and that only the larger albuminoid and \(a\)-crystalline molecules, which have molecular weights of 800,000 or more\(^22\) and which represent 45 per cent of the total protein,\(^23\) are retained by the impermeability of the capsule to large proteins.\(^19\) The changes in other lens components are consistent with this explanation. There are only traces of sugar alcohols in the aqueous of diabetic and galactose-fed rats.\(^10\) With the appearance of mature cataracts, the sugar alcohols suddenly disappear from the lens in accord with the concentration gradient. The aqueous level of glucose in diabetic rats is essentially the same as that of the plasma,\(^24\) whereas the lens concentration is considerably lower. With the appearance of mature cataracts, glucose suddenly increases in the lens in accord with the concentration gradient. Similarly, ATP and fructose, which are generated within the cell, suddenly decrease in concentration. The sorbitol space which was determined with radioactive sorbitol and is therefore independent of the sorbitol content of the lens provides an estimate of the extracellular space of the lens. With the appearance of mature cataracts it increases markedly. It seems fair, therefore, to conclude that the appearance of mature cataracts is associated with a disruption of the fiber membranes and that the remaining proteins are retained in the lens by the lens capsule.

Under physiologic conditions the impermeability of the cell membrane to protein and the presence of higher concentrations of protein within the cell than exist in the extracellular fluid would lead to osmotic swelling. This is counterbalanced,\(^25\) however, in the living cell by the active extrusion of sodium ions and the relatively high concentration of this ion in the extracellular fluid. If the fiber membranes are disrupted after the appearance of mature cataracts, then lens hydration would be dependent on the permeability of the capsule. Since the capsule retains large protein molecules but is freely permeable to cations, the usual balance is upset and conditions are right for lens swelling. The sudden increase in the hydration index after the appearance of mature cataracts is consistent with this explanation.
It has been suggested\(^2\) that the vacuoles which are noted soon after galactose feeding is started are the result of osmotic swelling and eventual rupture of fibers following the accumulation of sugar alcohols. The continuation of this process would eventually lead to massive fiber membrane disruption, such as that which has been described as accompanying the appearance of mature cataracts. If this hypothesis is correct, then the course of the changes during the period before the appearance of mature cataracts should reflect the changes that are occurring. The contribution of disrupted fibers to the resultant situation can be estimated by the levels that exist after the appearance of mature cataracts. For instance, the hydration index is more than tripled after the appearance of a mature cataract and the new level is maintained steadily for at least 10 days. This should be reflected in the hydration index during the period of galactose feeding. If the wet weight is corrected for the weight of the water associated with swelling due to sugar alcohol,\(^8\) and the dry weight is similarly corrected for the presence of sugar alcohol, then a corrected hydration index may be calculated. Now if the dry weight of the lens is made up of substances that are found in the fibers of normal lenses, the hydration index would be about 2.6. If, however, some of the dry weight is due to the presence of substances that are associated with disrupted fibers having a hydration index of 8.0, then the resultant hydration index should be appreciably above the normal level. A similar increase in the resultant hydration index would be obtained if there were an accumulation of an unknown small molecule, similar to that of the sugar alcohols, within the lens. The results of a calculation of this type are shown in Table I. The ratio of the corrected hydration index of the galactose-fed rats to the hydration index of normal rats increases steadily for 14 days and then the ratio returns toward unity. The latter observation has been made repeatedly with 25- to 27-day-old rats that are started on a 35 per cent galactose diet. In older rats\(^4\) the hydration due to sugar alcohols more than accounts for the swelling, and the ratio of hydration indices is not increased. The same is true for lenses from rats with diabetes. Therefore, it is felt that the observed increase in the ratio of hydration indices is possibly caused by the presence in the lenses of young rats on a galactose diet of some unidentified small molecule which does not accumulate in older rats.

Although there may be a minor disruption of lens fibers during the period before the appearance of mature cataracts, the return of the ratio of hydration indices to normal levels in young rats and the lack of an increase in older rats, plus the failure of the extracellular space to show marked changes from the average, do not support the concept that there is a steady disruption of fibers during the period before the appearance of mature cataracts.

The mechanism that is responsible for the disruption of fiber membranes is not known. It is known that the process becomes irreversible on about the tenth day of galactose feeding and that mature cataracts will occur in spite of a lowered dulcitol level.\(^26\) It is also known that

### Table I. The hydration index of lenses from galactose-fed rats, corrected for swelling due to dulcitol, compared with the hydration index of lenses from normal rats

<table>
<thead>
<tr>
<th>Days on diet</th>
<th>Galactose-fed hydration index (corrected*)</th>
<th>Normal hydration index</th>
<th>Ratio A/B</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>20</td>
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\*Corrected hydration index equals wet weight in milligrams (milligrams of dulcitol per lens \(\times 16.5\)) divided by the dry weight of the lens in milligrams (milligrams of dulcitol per lens).
galactose cataracts can be delayed with appropriate diets. The relationship between the disruption of the fiber membranes and the appearance of mature cataracts is not known.

REFERENCES