A biochemical evaluation of a cataract induced in a high-glucose medium

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Rabbit lenses were incubated in low- and high-glucose media in an attempt to evaluate the role of the sorbitol pathway in the production of this sugar cataract. The aldose reductase inhibitor, 3,3-tetramethylene glutaric acid (TMG), was employed to block sorbitol formation. Exposing lenses to high glucose leads to an initial linear increase in sorbitol content and lens water. During the first four days of incubation, lens swelling occurs in response to intracellular sorbitol accumulation. Swelling renders cell membranes more permeable to sodium and potassium. During the first four days in high glucose, the swollen lens is able to compensate for this increased leakiness to sodium and potassium, presumably through increasing cation pump activity. Thus there is no increase in the absolute cation level. However, on or about the fifth day in high glucose, the capacity of this compensatory mechanism is exceeded and abrupt changes in lens cations occur. Lens sodium rises and lens potassium falls; the net result is an increase in total cations. At a somewhat later stage, the insulin space of the lens increases as lens fibers rupture and/or become more permeable to insulin. The addition of TMG to the high-glucose medium practically abolishes sorbitol accumulation; it depresses lens swelling, preserves normal cation balance, and maintains lens clarity and transparency for eight days. This suggests that all of the aforementioned changes are interrelated and also emphasizes the primary role played by aldose reductase in the initiation of the entire sequence of cataractous change.

Key words: crystalline lens, cataract, glucose, sorbitol, sodium, potassium, metabolism, aldose reductase, 3,3-tetramethylene glutaric acid, enzyme inhibition, cell membrane permeability, lens swelling, lens hydration, extracellular space.

Recent investigations have established the role of the sorbitol pathway in the genesis of the experimental galactose cataract. Although normally present in the lens, the pathway assumes significance pathologically only when the lens is exposed to abnormally high levels of sugar. The individual steps of the pathway are illustrated in Diagram I.

Only in the presence of a high aldose concentration (i.e., xylose, galactose, glucose) will appreciable polyol formation occur. The Michaelis constants of aldose reductase for these sugars are exceptionally high; this explains the inactivity of this

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Diagram I.

pathway under normal conditions. Exposing a lens to a high-galactose medium in vitro or feeding a rat a high-galactose diet results in the intracellular accumulation of dulcitol by the lens. Le Fevre and Davies and Wick and Drury have shown that polyols do not readily diffuse through biological membranes. As dulcitol accumulates, it renders the intracellular milieu hypertonic, and the resulting osmotic imbalance brings water into the lens fibers. Swelling in response to intracellular dulcitol accumulation renders the cell membranes more permeable to cations; sodium influx leads to more swelling and fiber disruption. These changes are fundamental to the formation of the galactose cataract.

By using an aldose reductase inhibitor to block dulcitol formation, one may be able to prevent the formation of a galactose cataract. Recently the aldose reductase inhibitor, 3,3-tetramethyleneglutaric acid (TMG), has been employed to prevent dulcitol formation in lenses incubated in high-galactose medium. It was successful in completely preventing cataract formation in vitro.

Many investigators have implicated the intracellular polyol accumulation in the genesis of sugar cataracts. van Heyningen was the first to document the presence of sugar alcohol in cataractous lenses of galactosemic and xylosemic rats. Patterson and Bunting and Kuck have demonstrated high levels of sorbitol in lenses of diabetic rats and rabbits. In the light of this evidence, a more detailed evaluation of the role of the sorbitol pathway in cataract formation in lenses exposed to high glucose seemed indicated.

It has been the purpose of this study to devise a method satisfactory for a study in vitro of the evolution of the glucose cataract of the rabbit lens. With the use of this method and the aldose reductase inhibitor, TMG, an evaluation of the role of the sorbitol pathway in the production of this sugar cataract has been made.

**Methods and materials**

Albino rabbits that weighed from 1½ to 2 pounds were killed by means of air embolus. Their eyelids were painted with tepid petrolatum to prevent fur from adhering to the globe during enucleation. The lenses were removed via a posterior approach and, after the vitreous body had been stripped off, were incubated in 20 ml. of incubation medium contained in a special incubation flask (see Fig. 1). This flask is a modification of that employed by Lambert and Kinoshita. It remains stationary in the water bath, thereby eliminating any lens trauma due to movement of the apparatus. Both the gaseous atmosphere and the incubation medium can be changed aseptically without opening the flask, thus minimizing the chance of exogenous contamination. The medium is composed principally of Medium 199 without phenol red (Microbiological Associates, Bethesda, Md.) to which additions are made so that the final concentration of bicarbonate is 34 mM., and of calcium ion 2.5 mM. In the low-glucose (LG) control medium the glucose concentration is 5.5 mM., and the fructose concentration is 30 mM. In the high-glucose (HG) experimental medium the glucose concentration is 35.5 mM. Composition of the basic medium has been described. The tonicity of both the LG and HG media is 290 ± 1 milliosmolal as determined by an osmometer obtained from the Advanced Instrument Co. (Newton Highlands, Mass.). In paired lens experiments one lens is incubated in the control medium containing 30 mM. fructose and the other in the 35.5 mM. glucose medium. The incubations lasted a maximum of 10 days, after which the lens was removed from the culture flask, rolled on filter paper to remove adherent water and zonules, and weighed in a glass homogenizer. With the use of a Teflon grinder and either 10 per cent trichlo-
Fig. 1. Photograph of assembled incubation apparatus (left). Close-ups of the nylon lens bed (right). The lens rests on a piece of dialysis tubing held between the upper and lower parts of the lens bed. The lateral opening facilitates exit of gases trapped beneath the apparatus. The entire bed is immersed in 20 ml of incubation medium. The medium can be added or withdrawn sterilely through the attenuated pipette supporting the lens bed.

Roacetic acid (in deionized water) or ZnSO$_4$·Ba(OH)$_2$, lenses were homogenized and the homogenates were spun in a Sorvall centrifuge. The supernatants were used for the chemical analyses. Hexitols were measured chemically according to the procedure of West and Rapoport$^{18}$ as modified by Faulkner.$^{20}$ Samples from lenses incubated with TMC were analyzed for sugars and hexitols by gas liquid chromatography (Hayman and co-workers$^7$ and Sweeley and co-workers$^{24}$) with an F and M Model 402 gas chromatograph. Both methods yielded similar results.

Sodium and potassium levels were measured by standard procedures by means of an Advanced flame photometer and an internal lithium standard.

The uptake of $^{86}$Rb and $^{24}$Na was studied by preincubating paired lenses in LG and HC medium free of $^{86}$Rb or $^{24}$Na. At the end of the incubation period, $^{86}$Rb or $^{24}$Na (obtained from Iso-serve Corp., Cambridge, Mass.) was added to the medium to give 30,000 ct. of $^{86}$Rb per minute per milliliter or 70,000 ct. of $^{24}$Na per minute per milliliter and the incubation continued for four hours. At the end of the four hours, both lens and medium were assayed for radioactive Rb or Na. All isotope measurements were made with a Tri-Carb Liquid Scintillation Counter, Model 314EX.

The rubidium run-out experiments were designed following the procedures described by Becker and Cotlier$^{25}$ and were conducted according to the details published in an earlier paper.$^8$

Inulin-carboxyl-$^{14}$C (New England Nuclear Corporation, Boston, Mass.) was used to estimate the lens extracellular space. Paired lenses were preincubated in LG and HG medium free of $^{14}$C-inulin. At the end of this incubation enough $^{14}$C-inulin was added to the medium to yield 70,000 ct. per minute per milliliter and the incubation was continued for 4 hours. Thoft and Kinoshita$^{11}$ have shown that $^{14}$C-inulin equilibrates with lens extracellular space within 75 minutes. Upon completion of the 4 hour incubation, both lens and
medium were analyzed for $^{14}$C, and results expressed as per cent inulin space. This was calculated as follows:

$$\text{Per cent inulin space} = \frac{\text{vol. inulin space per lens (ml.)} \times 100}{\text{wet weight of lens (Gm.)}}$$

TMG was obtained from Ayerst Research Laboratories (Montreal, Canada). A previous study has shown that TMG does not penetrate the lens readily. $10^{-5}$M TMG inhibits an enzyme preparation of lens aldose reductase by 90 per cent but, with an intact lens incubated with TMG, no inhibition occurred below $5 \times 10^{-5}$M. In the following experiments, the final concentration of TMG is $10^{-2}$M. A 709.8 mg. amount of TMG was added to 390 ml. of LG and HG medium and dissolution was hastened by heating the flask with hot tap water. After the TMG had dissolved, it was neutralized with Tris-Cl, and the solution equilibrated with 5 per cent CO$_2$-95 per cent air for 30 minutes. The tonicities of the LG and HG media with TMG were identical, ranging between 290 and 293 milliosmolal. Incubations were set up in the standard manner.

**Results**

An evaluation of the suitability of the lens-culture procedure for long-term culture of 150 to 200 mg. of rabbit lenses was made. Paired lenses were incubated in a control medium containing 5.5 mM. glucose. One lens was incubated for 24 hours; it was then removed and analyzed. The other lens was incubated for varying lengths of time up to nine days, then removed and analyzed. The results indicated that rabbit lenses weighing approximately 150 to 200 mg. can be incubated successfully for nine days without significantly altering wet weight or sodium or potassium content. There is no significant loss of lens clarity; a posterior annulus appears after the fifth day corresponds in location to the ligament of Wieger which is ruptured during removal of the vitreous. The dry weight increases approximately 2.5 mg. during a nine-day incubation.

**Description of cataractous changes.** The effect of exposing lenses to high glucose was studied in vitro. One member of a lens pair was incubated in a LG control medium, and the other in a HG experimental medium. During a nine-day incubation, approximately 50 per cent of the control lenses remained crystalline, 30 to 50 per cent remained crystalline but manifested a posterior annulus, and 0 to 30 per cent developed varying degrees of posterior opacification.

Lenses in a HG medium manifested a reproducible pattern of cataractous change which is presented below:

**Day** **Description**

1. The lens is usually crystalline; rarely a posterior annulus is present
2. Anterior equatorial haze emerges and the posterior annulus is accentuated
3. Clouding of, and sector-shaped spicule formation are noted at the posterior pole; 20 to 40 per cent of lenses have a clear posterior pole
4. The equatorial opacity extends to straddle the equator and also involves the anterior aspect of the lens; practically all lenses show a posterior pole opacity
5. The changes of Day 4 continue; a few lenses still show clear anterior and posterior poles
6. There is intensification of the anterior and posterior opacity
7. Many lenses show separation of the anterior capsule and epithelium from the underlying cortex
8. All lenses show confluent anterior and posterior opacification; if the lens is sliced open now, the nucleus is clear
9. Lens rupture occurs in a majority of lenses; this is evidenced by bleb formation
10. The glucose-cataract consistently progresses through an early stage of equatorial opacification and gradual sequential opacification of the posterior and anterior poles; later there is separation of the anterior capsule and epithelium from the underlying cortex and ultimately lens rupture. Morphologically there are striking similarities between the cataract which we have produced and the cataract of the alloxan-diabetic rabbit. The cataract of the diabetic rabbit shares many morphological characteristics with the human juvenile diabetic cataract. Thus the rabbit lens cultured in vitro appears to be an excellent model for the study of this cataract.

**Weight changes.** The lenses incubated
in HG medium manifest a linear net increase in wet weight during the first five days of incubation. As mentioned previously, no wet weight changes occur in LG medium. After the fifth day the gain in wet weight is nonlinear: it peaks at seven days and then decreases (see Fig. 2). Since the dry weight of lenses incubated in HG medium is not significantly different from that in LG, the difference in wet weight reflects a change in lens water. Therefore almost all of the weight increase during the first seven days is due to an increase in the volume of the aqueous phase.

**Sorbitol data.** Exposing the lens to high glucose leads to a linear increase in wet weight and a linear net increase in sorbitol during the first four days of incubation (see Fig. 2). Thereafter the sorbitol con-

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![Fig. 2. Net sorbitol accumulation and net increase in the wet weight of lenses incubated in a high-glucose medium. The net values are calculated as the difference between values of experimental and control lenses, both having been adjusted to correspond to a lens weighing 175 mg. Each point represents an average of results obtained from at least eight paired incubations.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933003/)
tent achieves a brief plateau at 11 µmoles per lens (corresponding to 62.8 µmoles per gram of tissue), and on the sixth day begins to decline.

An estimate of the ratio of total sugar (µmoles per lens) to the average change in wet weight (grams per lens) was made (see Table I). The results suggest that the ratio during the first four days is roughly constant and equal to the tonicity of the incubation medium, and that the increase in lens water during the first four days is due to the movement of water into lens fibers in response to the osmotic imbalance caused by the intracellular accumulation of sorbitol.

One aspect of our investigation may lead to misleading interpretation. Since the metabolism of glucose by the lens via the sorbitol pathway is accelerated in the HG medium, it is surprising that the fructose levels in these lenses incubated in the experimental medium are not higher than those observed in the control medium. To preserve lenses for long periods in the control medium it was helpful to add 30 mM fructose in place of the additional 30 mM glucose in the HG medium. Fructose was preferable to an equivalent amount of NaCl. Thus the high fructose level in control lenses reflects accumulation consequential to the high concentration of fructose in the medium and is much higher than normally found.

In a lens freshly removed from the rabbit eye or one incubated in a medium without added fructose the ketose level is about 0.2 µmoles per lens. When this value is compared to those obtained in HG medium it does appear that fructose is synthesized presumably through the sorbitol pathway. The fructose level seems to reach a plateau when the concentration reaches about 8 µmoles per gram of lens.

**Sodium and potassium data.** Since electrolyte changes could also have profound osmotic effects, this parameter was also examined in lenses exposed to high glucose. During the first three days of incubation in a HG medium, there is no significant change in the sodium content. On the fourth day, a significant rise in lens sodium occurs—from 2.4 to 6.5 µEq per lens (p less than 0.01). Thereafter the lens sodium remains elevated. There is a general decline in the potassium content of both control and experimental lenses during a nine-day incubation (see Fig. 3). However, the difference between control and experimental lenses is not significant until the sixth day when p is less than 0.02. At nine days the difference is significant below the 1 per cent level.

If the total cations (that is, the sum of the Na and K ions) are expressed as µEq per kilogram of dry lens weight, there is no significant change in the sodium content. On the fourth day, a significant rise in lens sodium occurs—from 2.4 to 6.5 µEq per lens (p less than 0.01). Thereafter the lens sodium remains elevated. There is a general decline in the potassium content of both control and experimental lenses during a nine-day incubation (see Fig. 3). However, the difference between control and experimental lenses is not significant until the sixth day when p is less than 0.02. At nine days the difference is significant below the 1 per cent level.

If the total cations (that is, the sum of the Na and K ions) are expressed as µEq per kilogram of dry lens weight, there is no significant change in the cation content of the lens during the first four days. On the fifth day, there is an abrupt increase in lens Na. The net result of this change is an absolute increase in the total cation
Fig. 4. Lens cation changes during incubations in low- and high-glucose media. Values have been adjusted to correspond to a 175 mg. lens and are expressed on a lens water basis (left) to reflect change in cation concentration and on a dry weight basis (right) to reflect change in the absolute amount of cation. Each column represents an average of results obtained from at least eight paired incubations.

content of the lens on or about the fifth day in HG medium (see Fig. 4).

If the cation results are expressed in terms of μEq per kilogram of lens water (reflecting cation concentration), there appears to be a gradual drop in K concentration and total cation concentration during the first four days (see Fig. 4). The abrupt increase in the amount of lens Na on the fifth day is reflected in a transient rise in total cation concentration. However, after the fifth day, the cation concentration continues to decrease gradually.

Inulin space data. It has been shown that the inulin space of the lens is a reasonable approximation of the extracellular space. During the first five days of incubation there is no significant difference between the inulin space of control and experimental lenses (see Fig. 5). On the fifth day a rapid expansion of the inulin space begins—from 3 to 22 per cent. In the absence of a concomitant rapid increase in lens wet weight, most likely, this represents entry of inulin into the intracellular space through leaky cell membranes. It could also indicate rupture of lens fibers and a consequent marked increase in the extracellular space. Presumably the intracellular compartment was free of inulin prior to the fifth day.

Fig. 5. Changes in the inulin space of lenses incubated in low- and high-glucose media. The inulin space, a reasonable approximation of lens extracellular space, is calculated as follows:

\[ \text{Per cent inulin space} = \frac{\text{vol. inulin space per lens (ml.)} \times 100}{\text{wet weight of lens (Gm.)}}. \]

Each point represents an average of results obtained from at least six paired incubations.

Cation permeability data. Concomitant with the measurement of the inulin space with \(^{14}C\)-inulin, a study of the rate of \(^{23}Na\) uptake was also made. Results showed a significant biphasic increase in the c.p.m.
of $^{24}$Na per lens (see Fig. 6). There was a rapid increase during the first five days followed by an even more rapid increase in the latter half of the nine-day incubation. In the absence of a significant rise in the absolute amount of lens Na during the first four days of incubation in high glucose, this increase in $^{24}$Na content represents an increase in the rate of Na entry into the intracellular compartment of the lens. However, the lens appears capable of maintaining a normal absolute amount of lens Na by in some way compensating for the increased Na entry.

$^{86}$Rb was also used to study permeability characteristics of the swollen lens. The Na-K pump was blocked with $10^{-4}$M ouabain after preloading control and experimental lenses with $^{86}$Rb; then the rates of run-out of $^{86}$Rb for control and experimental lenses were compared. The results show a progressive increase in the rate of $^{86}$Rb run-out in experimental lenses from 38 per cent at day 1 to 97 per cent at day 7. The control lenses manifested an unchanging rate of $^{86}$Rb run-out.

$^{86}$Rb uptake by lenses incubated in high glucose was measured after exposing them to a $^{86}$Rb medium for four hours. The $^{86}$Rb uptake in experimental lenses was higher than controls for the first four days (see Fig. 7). This excess decreased gradually so that on the fifth day experimental lenses were accumulating less $^{86}$Rb than controls. In the presence of a gradually decreasing absolute lens K, this initial elevation of $^{86}$Rb in experimental lenses represents an increased rate of Rb turnover in the lens. It has been shown that Rb and K are handled identically by the Na-K pump in the lens; therefore the increase in Rb turnover also represents an increase in the rate of K turnover. Thus the increased membrane permeability to cations in the swollen lens is initially compensated by an increased rate of K uptake and Na extrusion by the lens.

**Incubation with TMG.** The inclusion of $10^{-2}$M TMG in the HG experimental medium had a remarkable effect on the pattern of lens change during long-term incuba-
Throughout the eight-day incubation, a majority of lenses in LG and HG media maintained the crystalline appearance of a normal lens. None of the lenses mimicked the pattern of cataract evolution found in the absence of TMG. A minority of lenses manifested a fine, posterior surface annulus, and a few lenses also showed a fine granularity at the anterior pole. The incidence of these changes was identical in both control and experimental lenses and was probably not related to the effects of high glucose. It was our impression that control lenses incubated with TMG preserved a more perfect crystalline appearance than those without TMG. Lenses incubated in HG medium with TMG gained only 6.6 mg. in lens water during an eight-day incubation compared to a gain of 50 mg. in lens water in the absence of the inhibitor. This represents only 13 per cent of that which occurs in identical media without TMG.

TMG does not impede the entry of glucose into the lens; the amount of glucose was 13 times higher in the experimental lenses than in the control lenses throughout the incubation (see Fig. 8).

There was no significant difference between the sorbitol content of control and experimental lenses (see Fig. 9). The sorbitol content of both groups increased only slightly during the eight-day incubation from 0.6 to 2.0 μmoles per lens. Compared to the results obtained in incubations without TMG, this represents a significant inhibition of sorbitol production. This concentration of TMG is known to inhibit the production of dulcitol by 90 per cent in the intact lens, while exerting no effect on lens sorbitol dehydrogenase.

An equally remarkable effect of TMG on the evolution of the sugar cataract was its ability to preserve normal cation balance. Throughout the eight-day incubation, Na and K levels of lenses in high glucose did not differ significantly from those in low glucose, nor did they differ significantly from normal lens values for Na and K (see Fig. 10).

Discussion

It has been the purpose of this study to evaluate in vitro the importance of the sorbitol pathway in cataract formation in rabbit lenses exposed to high glucose. A review of the data establishing the importance of this pathway in galactosemic cataracts has been presented. A detailed description of the cataract in the alloxan-diabetic rabbit by Bounds and colleagues and Waters has emphasized the pattern of equatorial vacuoles spreading to opacify the anterior subcapsular region. Morpho-
Fig. 10. Na and K content of lenses incubated in low- and high-glucose media containing 10^{-4}M TMC. There is no significant difference between the cation levels of experimental and control lenses at any time. The control column represents an average of individual controls of each incubation. Each column represents an average of results obtained from at least eight paired incubations.

logically the cataract which we have observed in vitro closely resembles the cataract described by Bounds and colleagues and shares many important features with the galactosemic cataract described by Kinoshita.

The increase in wet weight of lenses incubated in 35.5 mM glucose is linear for the first five days and appears to be due almost exclusively to an increase in intracellular lens water. Concomitant measurement reveals no significant change in the dry weight of the lens nor in the size of the inulin space. This increase in intracellular fluid could be due to the intracellular accumulation of electrolytes or of a nonpermeating metabolic end product. In the case of the galactosemic cataract a similar gain in wet weight has been shown to be due to the intracellular accumulation of dulcitol, the sugar alcohol of galactose. It has been shown that sugar alcohols do not readily diffuse through biological membranes. Similarly in the glucose cataract, sorbitol, the sugar alcohol of glucose does seem to create an osmotic imbalance favoring the movement of water into the lens cells. During the first four days, there is no significant increase in the amount of Na or K per lens; on the other hand, a linearity of the increase in both wet weight and sorbitol has been established. The evidence strongly suggests that there exists a direct relationship between the sorbitol accumulation and lens swelling.

During the first four days in high glucose the swelling of the lens in response to the intracellular accumulation of sorbitol constitutes a stress to the cell membrane. This results in the progressive increase in the permeability to cations as the period of exposure to high glucose is lengthened. However, despite this increase in permeability, there is no significant change in the absolute level of Na or K, indicating that the lens has the capacity to compensate for the leakiness in the lens membranes. The increase in K leakage and Na entry is compensated for by an increase in cation pump activity. This adjustment helps to preserve the normal cation levels and is reflected by the increased turnover rates of cations. The increases in uptake of ^{86}Rb and ^{24}Na, without a change in the cation level, are consistent with this conclusion. Exposure of the lens to high galactose in vivo and in vitro has also been shown to increase the rate of cation turnover.

After four days of incubation in high glucose, a marked alteration in several lens parameters begins. The cataract progresses from equatorial and minimal posterior clouding to extensive anterior and posterior cortical opacification. Ultimately lens rupture occurs. These drastic visible manifestations are undoubtedly related to an alteration in the composition of osmotically active components. The total cation level, which had remained essentially unchanged for the first four days, begins to increase rapidly, mainly due to a rise in sodium. Apparently the cation pump is no longer able to keep pace with the increase in permeability to cations, and the total cation content rises. With the increase in electrolytes, osmotic change is no longer solely...
regulated by the sorbitol level; the elevation in electrolytes also contributes to the lens swelling. This marked electrolyte change is also reflected in the inability of the lens to accumulate $^3$H Rb or exclude $^2$HNa at the normal rate. At a somewhat later period, the inulin space begins to expand. The experiments with isotopic cations suggest that the increase in permeability to cations precedes the expansion of the inulin space. The increase in the uptake of inulin may be the result of disruption of lens fibers in some areas, or actual penetration of inulin into some of the fibers. The gradual decline in lens sorbitol during this period may also be a reflection of the increase in permeability. The rate of sorbitol synthesis may be unchanged, but accelerated diffusion out of the lens at this late stage could explain the depressed polyol level. All the findings observed in this stage of the cataract seem to point out that major changes have occurred in the lens permeability barrier.

The most convincing data implicating the sorbitol pathway in the production of cataractous changes in lenses exposed to high glucose are revealed in the experiments with the aldose reductase inhibitor, TMG. The addition of TMG to the HG medium practically abolishes the sorbitol accumulation; it effectively prevents lens swelling and maintains the clarity and transparency of the lens for eight days. Further supporting the contention that all these changes are interrelated is the fact that TMG also preserves normal cation balance throughout the incubation. Thus, these findings with TMG strongly suggest that, in a lens exposed to high levels of glucose, aldose reductase plays a primary role in the initiation of the entire sequence of cataractous change.

A basic aim of this study has been to design an organ culture system in which lenses could be maintained in their normal crystalline state for prolonged periods. We have not been completely successful in realizing this aim. Control lenses incubated for more than seven days, although clear, are beginning to undergo changes in cation balance and extracellular space. These changes undoubtedly influence the progression of cataractous change which has been observed in lenses incubated in high glucose. Studies are now in progress to determine better conditions under which control lenses can be maintained for longer incubations. With longer incubations it may be possible to clarify the changes in cation balance, protein content, and other lens parameters that occur in the later stages of this sugar cataract.

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