Mechanism of "hypoglycemic" cataract formation in the rat lens. I. The role of hexokinase instability

Leo T. Chylack, Jr.

Lenses from 100 gram albino rats remain clear and possess normal levels of Na+, K+, ATP, and hexokinase activity for 20 hours incubated in medium containing 12 mM glucose. Below 2.0 mM glucose, a cataract forms and there is an abrupt rise in lens Na+ and wet weight, a fall in lens K+, ATP, and hexokinase activity. The cataract is a thin lamellar opacity involving the anterior and posterior surfaces of the lens. If the lens is deprived of glucose for 48 hours, a nuclear cataract forms; the cortex between the superficial lamellar opacity and the nucleus being clear. This experimental cataract bears a striking resemblance to the hypoglycemic cataract seen in children. The thermal deactivation of hexokinase follows rapidly upon the depletion of its substrates (ATP and glucose) and is a primary factor leading to cataract formation. This was established by incubating the lens with 2-deoxyglucose, a competitive inhibitor of lens hexokinase. This compound blocks the entry of glucose into the glycolytic sequence. The cataract formed in its presence is identical morphologically and biochemically to that observed in a glucose-free medium. The effects of 2-deoxyglucose are prevented by increasing the glucose level; this rules out a direct toxic influence of 2-deoxyglucose and further supports the primary role of hexokinase thermolability in the etiology of this experimental cataract. This in vitro system appears to be an excellent experimental model for the study of the human hypoglycemic cataract.

Key words: hypoglycemia, neonatal, cataract, lens metabolism, hexokinase, thermolability.

The association of cataract with infantile hypoglycemia was first reported by Scheie, Rubenstein, and Albert in 1964. Since then, this association has been reported in varying detail by several authors. The mechanism of cataract formation in this disorder is unknown although several authors have assumed that the low blood sugar per se is causally related to the lens opacity. There is some evidence to support this assumption; glucose is the main substrate for energy yielding or conserving processes in the lens. The young lens is not able to sustain itself and becomes cataractous in the absence of glucose, even if adequate atmospheric oxygen is present. Glucose must be obtained from the aqueous humor since there is little, if any, gluconeogenic activity in the lens. The ability of the lens to sustain itself in a...
glucose-deficient environment by the aerobic metabolism of endogenous substrates is controversial and may vary with age. The young calf lens has been shown to be entirely dependent on glucose while the adult bovine lens may be able to sustain many metabolic functions by the oxidation of endogenous amino acids. Recognizing this central role of glucose in the homeostasis of the young lens, it then is essential to examine those enzymes involved in glucose metabolism by the lens. The most important of these enzymes is hexokinase (ATP-D-hexose-phosphotransferase [E.C. 2.7.1.1]), the so-called "pacemaker" of lens glycolysis. This enzyme is saturated at normal levels of aqueous glucose, raising the level of blood or aqueous glucose does not lead to increased glycolytic activity or lactic acid formation. Hexokinase is 70 to 1,000 times less active than other glycolytic enzymes in the lens. Lens hexokinase has been studied in detail in recent years. The possibility of a relationship of hexokinase to cataract formation in hypoglycemia was suggested by the finding of marked instability and inactivation of hexokinase at 37.5°C in the absence of glucose. It is the purpose of this paper to report the results of a study of the formation of cataract in lenses incubated in glucose-deficient media and the role of hexokinase instability in the formation of this cataract.

**Methods**

Lenses from 100 gram, male, albino rats (Charles River Breeding Laboratory, Wilmington, Mass.) were removed atraumatically from the enucleated globe and incubated with the vitreous body attached in 4.0 ml. of incubation medium in a 10 by 35 mm. plastic Petri dish (Falcon Plastics, Oxnard, Calif.) in a water-jacketed incubator (National Appliance Company, Model 3321, Portland, Ore.) containing an atmosphere of 5 per cent CO₂ - 95 per cent air and 100 per cent humidity. The incubation medium was based on a medium devised by Kinoshita and Obazawa-26 and contained (per 100 ml.): TC-199 (without glucose or phenol red, Grand Island Biological Company, Grand Island, N. Y.): 64.0 ml.; bicarbonate buffer (gram per liter NaHCO₃: 7.650; KHCO₃: 0.368; NaCl: 0.7431; KCl: 0.08533): 25.6 ml.; distilled water: 10.4 ml.; glucose (0 to 12 mM): 0 to 216.4 mg.; sorbitol (variable depending on the amount needed to make the final osmolarity of medium 290 ± 2 mOsm. per liter); fetal calf serum (GIBCO No. 614): 5.0 ml.; penicillin G (50,000 U. per milliliter): 0.02 ml.; streptomycin (5.0 Gm. per 9.0 ml.): 0.025 ml. When present in the medium, the 2-deoxyglucose concentration was 20 mM, and this replaced an equimolar amount of sorbitol. The medium was sterilized with a Millipore apparatus (Millipore Corporation, Bedford, Mass.) and then gassed with 5 per cent CO₂-95 per cent air for 30 minutes at 37.5°C. The final osmolarity of the medium was 290 ± 2 mOsm. per liter.

In this medium, lens pairs were incubated for 20 hours at 37.5°C in control medium (12.0 mM glucose) or experimental medium (0 to 6.0 mM glucose). Upon completing the incubation, the lenses were examined and photographed and then rolled on filter paper to remove the vitreous body. The wet weight was measured in a ground-glass homogenizer (Tenbroeck, VITRO, VWR Scientific Catalog No. 62400-493) and then homogenized in either 3.0 ml. of iced distilled water (for ATP assay), 2.0 ml. of 10 per cent trichloroacetic acid (for cation assays), or 0.5 ml. of 0.1 M Tris-C1 [Tris (hydroxymethyl) aminomethane] buffer, pH 7.40, at 5°C for hexokinase assay.

The ATP assay was a modification of the basic luciferin-luciferase fluorometric method devised by Strehler and Totter. Sodium and potassium assays were performed on the supernatant fraction of the TCA homogenate using a Perkin-Elmer Flame Photometer, Coleman Model 51.

The soluble, insoluble, and latent hexokinase activities of lens hexokinase were measured according to techniques previously described on a Spectrophotometer, Model 2400-2 (Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

Enzyme activities are expressed as a function of the volume of the enzyme preparation (A OD₅₅₀ per 5 minutes per 0.10 ml.); this is permissible since there was less than a 3 per cent difference in the protein content of the lens incubated in 0 mM glucose and that incubated in 12.0 mM glucose. Therefore, enzyme activity expressed as a function of volume is directly proportional to tissue-specific activity.

Protein was measured by the Lowry method.

Glucose was measured according to the ultramicro adaptation of the Glucostat method (Worthington Biochemical Corporation, Freehold, N. J.).

All enzymes except luciferin-luciferase (Sigma Chemical Company, St. Louis, Mo.) were obtained from Boehringer Mannheim Corporation,
Fig. 1. Comparison of fresh, unincubated lenses with fellow lenses incubated for 20 hours at 37.5°C in medium containing 12 mM glucose. The brackets indicate the standard error of the mean and the parentheses include the number of lens pairs studied. One hundred percent lens clarity indicates that both the fresh and incubated lenses were crystal clear and indistinguishable under a dissecting microscope.

New York. All other reagents were reagent grade.

Results

Control medium. The adequacy of our incubation system to maintain the lens in a nearly normal condition was evaluated by comparing the clarity, wet weight, ATP, Na+, and K+ levels, and hexokinase activity of fresh, unincubated lenses with similar values in fellow lenses incubated for 20 hours. Several concentrations of glucose were tried; 12.0 mM glucose proved to be the best (Fig. 1). These lenses remained crystal clear and maintained normal or near normal values for the parameters studied. This incubation system is excellent for short-term rat lens incubation.

Glucose deprivation. Paired lenses were incubated for 20 hours in medium containing either 12.0 mM glucose or 0, 0.5, 1.0, 2.0, 4.0, 5.0, and 6.0 mM glucose. The effect of such glucose deprivation on lens clarity, wet weight, ATP, Na+, K+, and hexokinase activity was measured. The lenses in low glucose medium remained crystal clear with glucose concentrations as low as 2.0 mM. At 1.5 mM glucose, a very faint, thin, diffuse, ground-glass type of opacification appeared in the anterior and posterior lens surfaces. This opacity appeared denser and thicker as the glucose concentration approached 0 mM. If the lens is similarly exposed to a glucose-free medium for 48 hours a nuclear opacity is seen in addition to the superficial lamellar opacity. The cortex between the nucleus and the superficial opacity remains grossly clear.

The ATP content of the lens drops below the normal, fresh lens value after 20 hours in 2.5 mM glucose medium. In 0.5 mM glucose medium, there is no detectable ATP after 20 hours (Fig. 2).
The wet weight of the lens does not increase until the concentration of glucose in the medium drops to 1.0 mM, but below this level, the gain in wet weight is marked and abrupt (Fig. 2). This was clearly evident by the swollen appearance of the cataractous lenses.

Accompanying this abrupt increase in wet weight is a sixfold increase in lens Na⁺ and a fourfold decrease in lens K⁺ (Fig. 3). Undoubtedly, this is a manifestation of the total decompensation of the ATP-dependent Na⁺ - K⁺ "pump."

Beginning at 2.0 mM glucose, there is a loss of total hexokinase activity compared to the control (12.0 mM glucose) lenses. This loss continues precipitously as the glucose level in the medium is lowered further, so that at 0 mM glucose, 82 per cent of the hexokinase activity has been lost. There is loss of both soluble and insoluble hexokinase; this is reflected in the relatively stable percentage of soluble hexokinase (Fig. 4). The latent hexokinase appears to be more resistant to thermal deactivation; as the glucose concentration in the medium drops from 12.0 mM to 0.0 mM, the per cent of total hexokinase in the latent form increases from 12.0 per cent to 48 to 79 per cent. That the loss of hexokinase activity is not due to the formation of an inhibitor was shown by experiments mixing low activity with high activity preparations of hexokinase and obtaining the predicted arithmetical sum of the two activities. To further confirm this loss of hexokinase activity as a thermal effect in a glucose-free system, rather than merely the effect of glucose deprivation alone, paired lenses were incubated in either 0.0 mM or 12.0 mM glucose for 20 hours at 0°C. At the end of such an incubation, there was no loss of enzyme activity in either medium, and the amounts of total, soluble, and insoluble hexokinase were also identical.

To be sure that the aforementioned changes were not due to complete glucose deprivation occurring after rapid depletion of the glucose in the medium, the glucose in the 0.5 mM medium was measured and

---

**Fig. 3.** The effect of glucose deprivation on rat lens Na⁺ and K⁺ levels. Between 12.0 mM and 1.0 mM glucose concentration there is no significant difference between experimental (< 12.0 mM glucose) and control (12.0 mM glucose) lenses. The dotted line indicates the mean value obtained in assays of fresh lenses. Each point is plotted with its standard error of the mean and represents the mean of at least six lens pairs.

**Fig. 4.** The total, soluble, and insoluble activity of rat lens hexokinase as a function of glucose concentration in a 20-hour incubation. Each point is plotted with its standard error of the mean and represents the mean of at least six lens pairs.
found to be approximately normal after a 20-hour incubation.

**Time-course of cataract formation.** To establish the rate at which changes in wet weight, hexokinase activity, and ATP content occur in the lens in a glucose-free medium, a time study was done (Fig. 5). During the first four hours of glucose deprivation, there occurs a 70 per cent drop in lens ATP. It has been shown\textsuperscript{26, 27} that the half-life of lens glucose is approximately 30 minutes. Therefore, after four hours in a glucose-free environment, the lens glucose must be reduced to approximately 4 per cent of its value in the fresh lens. Therefore, during this four-hour period, the levels of two of the substrates of hexokinase (ATP and glucose) are
drastically reduced. Both of these substrates stabilize lens hexokinase at 37.5°C.

It is not, therefore, surprising to see rapid deactivation of soluble and insoluble hexokinase beginning after four hours of complete glucose deprivation (Fig. 6). After 12 hours of glucose deprivation, total lens hexokinase is reduced to 22 per cent of control levels. Occurring concomitantly with this loss of hexokinase activity is a pronounced rise in lens wet weight and presumable also lens Na⁺.

The appearance of the superficial lens opacity occurs at approximately 8 to 12 hours.

This sequence of changes strongly suggests a direct role of hexokinase instability in the causation of the "hypoglycemic" cataract. However, to establish the primary role of this enzyme inactivation in cataractogenesis and to rule out the possibility that such enzyme inactivation is a secondary manifestation of cataractogenesis, an inhibitor of hexokinase, 2-deoxyglucose, was employed.

2-Deoxyglucose competes with glucose in the uptake mechanism in the lens; once within the lens cell, it is converted to 2-deoxyglucose-6-phosphate. In the process of this conversion, it competes with glucose for hexokinase and, as such, is a competitive inhibitor of this enzyme. The 2-deoxyglucose-6-phosphate neither inhibits hexokinase nor exerts any direct toxic influence on the cell. That such inhibition of soluble lens hexokinase occurs in the lens is shown in Fig. 7. At a concentration of 20 mM, there is 75 per cent inhibition of soluble lens hexokinase.

A series of lens incubations was then undertaken in which 20 mM 2-deoxyglucose was incorporated in the medium to act as a competitive inhibitor of lens hexokinase. Glucose, 2.0 mM, was used in the control medium, and higher levels of glucose were combined with 20 mM 2-deoxyglucose to evaluate the preventability of the deoxyglucose effect (Figs. 8, 9, and 10).

With 2.0 mM glucose in the medium, most of the lens parameters studied here were normal. The inclusion of 20 mM 2-deoxyglucose lead to the formation of a cataract identical in every way to that which formed in a glucose-free medium. There was a 23 per cent increase in wet weight, a 97 per cent decrease in ATP, a fivefold increase in lens Na⁺, an 82 per cent drop in lens K⁺, and an 80 per cent decrease in total hexokinase activity. All of these changes, including the lens opacity, were prevented by increasing the amount of glucose in the medium.

It is clear that the 2-deoxyglucose effect can be prevented by glucose; this eliminates a direct toxic effect of this compound on the lens as the basis for cataract formation. It also strongly suggests a primary role for hexokinase instability in the etiology of this experimental "hypoglycemic" cataract.
Figs. 8 through 10. Incubation of paired lenses in media containing 20 mM 2-deoxyglucose and varying amounts of glucose. Each bar represents the mean value of at least six lens pairs and is plotted with the standard error of the mean.

The mechanism of inactivation of hexokinase in the presence of 2-deoxyglucose is unknown. That it does not involve an increased thermolability is shown in Fig. 11. There is, in fact, a definite but incomplete stabilization of hexokinase at 37.5°C by 2-deoxyglucose. The more gradual slope of the initial deactivation phase in the preparation containing 2-deoxyglucose suggests that the stabilization is due to an effect on Type II hexokinase.

Discussion

The cataract seen in association with the neonatal, ketotic, and idiopathic types of hypoglycemia has been described as lamellar, nuclear, and posterior subcapsular. Many authors describe a "characteristic" type of lens opacity in this disorder—a lamellar opacity—or several such concentric lamellae—between which is sandwiched clear cortex. The nuclear cataract also has been described more as a zonular than a completely dense opacity. The ex-
Experimental "hypoglycemic" cataract in the rat lens bears a striking similarity, and no dissimilarities, to the human cataract. The clear cortex sandwiched in between the nuclear and superficial opacities is unmistakable in the 48-hour cataract. Even the nuclear opacity in many rat lenses appeared to be most pronounced in a zone adjacent to the cortex.

The lamellar nature of this cataract suggests the occurrence of an insult or series of insults during a distinct time period in early lens growth. The result of such an insult could be the formation of a layer of abnormal lens fibers which are later incorporated into the deeper layers of the lens. Such a layer would be separated from deeper and superficial cortex by clear lens cortex formed in the absence of any insulting stimuli.

The lamellar nature of this cataract suggests the occurrence of an insult or series of insults during a distinct time period in early lens growth. The result of such an insult could be the formation of a layer of abnormal lens fibers which are later incorporated into the deeper layers of the lens. Such a layer would be separated from deeper and superficial cortex by clear lens cortex formed in the absence of any insulting stimuli.

The results of this study suggest that hypoglycemia and the subsequent lowering of aqueous humor glucose could be such an insult to the lens. The time course of experimental cataract formation is sufficiently brief (12 hours) to allow lens damage to occur during a relatively brief period of hypoglycemia.

In the human, there is usually 16 to 18 hours between the last meal and the onset of hypoglycemic symptoms. The extremely high frequency (95 per cent) of blood sugars below 45 mg. per cent in full-term neonates four hours after delivery suggests that a single episode of hypoglycemia is a stress insufficient to cause cataract. Only 18 to 20 per cent of a series of childhood hypoglycemics had cataracts, and these were the most seriously affected children; this suggests that several hypoglycemic attacks are a necessary predisposition to cataract formation. It is very possible that this incidence is low due to the absence of complete detailed ophthalmological examinations of many of these children.

Cataracts have been formed in all types of childhood hypoglycemia; this suggests that the common feature is low blood sugar rather than some other change unique to a particular type of hypoglycemia.

The role of hexokinase instability appears to be central to the formation of lens opacity. Changes in ATP and lens glucose levels precede the enzyme deactivation, and the deficiency of ATP is most directly responsible for failure of the Na⁺-K⁺ active transport mechanism. However, it is...
the loss of hexokinase activity that precludes further ATP formation and deals to the lens an irreversible injury. That this cataract is irreversible after 12 hours of glucose deprivation will be the subject of a forthcoming publication.

The loss of ATP in the presence of 2-deoxyglucose and the incomplete prevention of this loss by increasing glucose levels (Fig. 8) even though hexokinase activity is back to normal levels suggest that ATP production only slightly exceeds utilization. It also could indicate damage to steps further on in the glycolytic sequence involved in ATP formation.

Although this study was not designed to evaluate the role of oxidative metabolism (respiration) in the rat lens, the rapid decompensation of cation gradients and the precipitous drop in lens ATP suggests that oxidative metabolism of endogenous or exogenous substrates was unable to maintain the aforementioned parameters of lens homeostasis. Kinoshita, Kern, and Merola17 studying the calf lens demonstrated a small but measurable contribution of aerobic mechanisms (in the absence of glucose) to the maintenance of lens Na+, K+, ATP, and protein synthesis. However, they concluded that glucose alone could sustain the young calf lens and could do so even in the absence of O2. Sippel27 concludes that no more than 33 per cent of the ATP (and probably less) is derived from oxidative metabolism of glucose in the rat lens. That the role of respiration in lens homeostasis may increase with age is suggested by Trayhurn and van Heyningen15 in their studies of bovine lens. They further demonstrated16 in the bovine lens an increased oxidation of amino acids in the absence of glucose. Although not quantitating the amount of ATP derived from such oxidation, they suggest that oxidation of amino acids is a source of energy in the adult lens. If present in the human adult, such oxidation of amino acids may protect the lens from hypoglycemic stress and explain why the typical hypoglycemic cataract does not develop in the adult human.

The hypothesis that a metabolic deficiency (i.e., ATP) and/or an enzyme deficiency can lead to cataract formation has stimulated abundant experimentation. However, a recent summary of this experimental work14 concludes that "to date, there is no evidence that cataract formation is initiated or even closely associated with depressed activity of any particular enzyme." The experimental studies herein reported, in contrast to the work cited by Kuck,14 have focused on a metabolic control point. It is at such a point that an enzyme deficiency might, and I believe does, lead to a significant disorder in lens homeostasis and cataract formation.

I would like to thank Mr. Frederick Schaefer for skilled technical assistance and Dr. Steven Fricker for the use of his computer.

REFERENCES
10. Dardenne, V., and Kirsten, G.: Presence and
25. Beckman Instruments Technical Bulletin No. 6073D.