
Mechanisms initiating cataract formation

Proctor Lecture

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Osmotic swelling is a common feature of many cataracts. Sugar cataracts and hereditary mouse cataracts are two types in which osmotic changes play a prominent role. In sugar cataracts, the initial swelling brought about by polyol accumulation leads to an imbalance in the pump-leak equilibrium. The pump mechanism becomes unable to keep pace with the leaky membranes. The marked increases in Na and Cl eventually results in Donnan swelling. In the hereditary mouse cataract, the imbalance of the pump-leak system appears to be initiated by a deficiency of Na-K ATPase, a component of the cation pump mechanism. The inefficiency in the pump mechanism results in Na retention and osmotic swelling.

Key words: sugar cataracts, hereditary mouse cataract, aldose reductase, aldose reductase inhibitor, deficiency of lens Na-K ATPase.

Factors involved in lens swelling

One common feature in many experimental cataracts is the dramatic change in hydration. This phenomenon has been observed in the following cataracts: sugar,¹ microwave,² hereditary mouse,³ ionizing radiation,⁴ naphthalene,⁵ and Triparanol.⁶ The initiating factor in the various forms of cataract may be different. In the end stages, however, the occurrence of the striking osmotic change due to an influx of sodium and chloride ions is apparent in all these cases. It becomes obvious that one

important mechanism in the lens is that which maintains the normal state of hydration. The lens volume is a balance of two opposing forces: one is the normal permeability characteristic of the lens membranes; and the second is the efficient cation pump that continually extrudes sodium ions and concentrates potassium ions. The intraocular fluids bathing the lens contain a high level of the Na and low K, while the cations in the lens have the opposite composition of high K and low Na. Thus, if allowed to come to equilibrium Na would enter and K would leave the lens. Because the lens membranes are impermeable to proteins the situation which allows for free exchange of cations would eventually lead to a Donnan type of swelling.⁷ In the lens, however, the cation pump mechanism linked to active metabolism normally prevents this from happening.

Kinsey⁸ describes the lens as a pump-leak system in which the levels of cations

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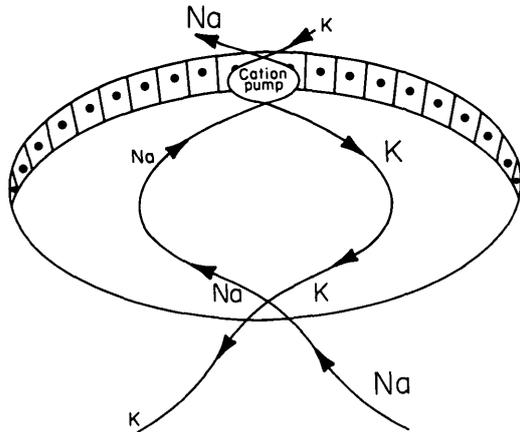


Fig. 1. Lens pump-leak system. Diagrammatic sketch depicting pump-leak system as described by Kinsey.⁸

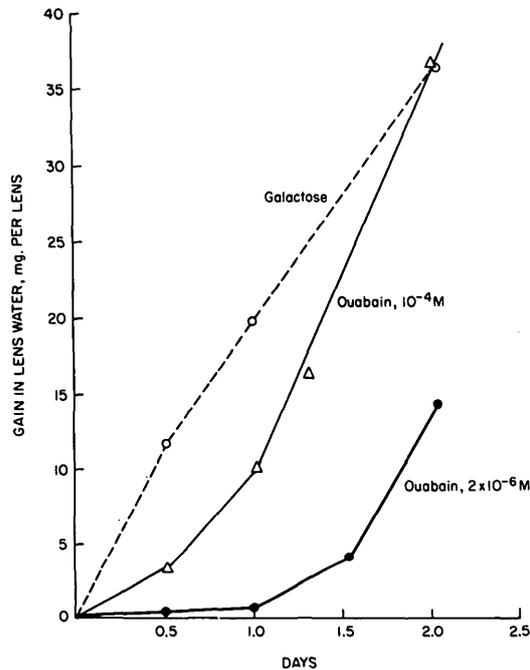


Fig. 2. Lens swelling by galactose and ouabain. Conditions for incubating rabbit lens in 30 mM galactose or in ouabain are described in previous reports.^{14, 16, 17}

are regulated by a balance between active uptake and passive diffusional processes. Kinsey's results suggest that Na enters principally across the posterior surface by passive diffusional uptake, it then diffuses anteriorly reaching the epithelium where it is actively extruded (Fig. 1). In contrast,

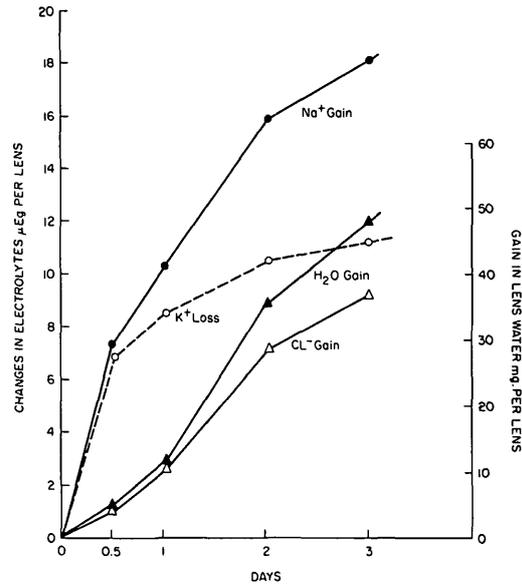


Fig. 3. Ouabain effects on the lens. Incubation conditions are described in Fig. 2.

K is actively transported into the lens at the anterior epithelial surface, diffuses posteriorly, and leaves the lens by diffusing across the posterior capsule (Fig. 1). Kinsey⁸ describes the movement of K and Na into and out of the lens as a through and through circulation (Fig. 1). These ions are constantly entering and leaving the lens and the forces that move the cations are the differences in chemical potential of the lens and the active transport mechanism which is primarily located in the epithelium. Maintaining the pump-leak balance is crucial to preserving the viability of the lens.

Ouabain effects

An example of upsetting the pump-leak balance can be shown by incubating the lens with ouabain, which is an inhibitor of the Na-K ATPase, the principal component of the cation pump mechanism. By blocking the cation pump mechanism with ouabain, the entry of sodium into the lens cannot be corrected by its extrusion at the anterior surface and eventually this situation results in swelling. However, the swelling is not immediate and a lag period, the length of which depends on ouabain

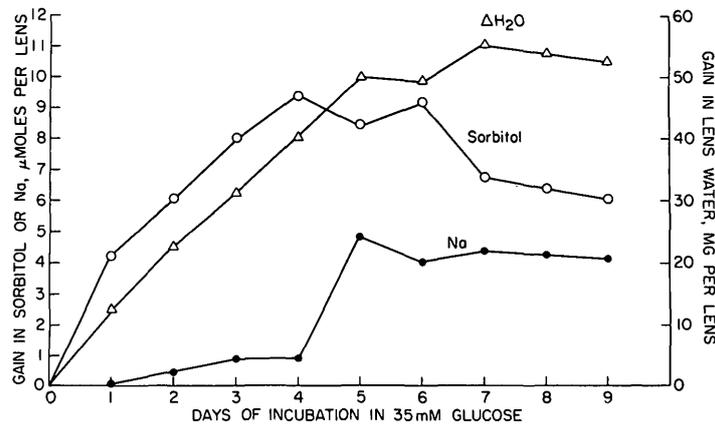


Fig. 4. The effect of a high glucose medium. Incubation conditions were described by Chylack and Kinoshita.¹⁶

concentration, is observed (Fig. 2). When the rabbit lens Na-K ATPase is 50 per cent inhibited by 2×10^{-6} M ouabain there is a substantial lag period, while at 10^{-4} M, where the inhibition is 90 per cent, the lag period is considerably shorter. The reason for the delay of swelling is that in the ouabain-inhibited lens the initial Na gain is matched by K loss, thus no net increase in cation occurs. When the entry of Na is no longer matched by K exit, a net increase in electrolytes results leading to an influx of H₂O. The initial 1:1 exchange of Na for K is illustrated in Fig. 3 which summarizes the results of incubating a rabbit lens in 10^{-4} M ouabain. When the gain in Na can no longer be compensated by a loss in K, chloride ions enter the lens to maintain electroneutrality and it is at this point that increase in hydration begins. As seen in Fig. 3, the curve representing the increase in lens water parallels the changes in chloride rather than those of Na or K.

Factors affecting the leak system

An example of affecting the pump-leak balance by the increase in lens cation permeability has been shown in the in vitro studies with either demercarium bromide (Humorsol),⁹ echthiophate iodide (Phospholine Iodide),⁹ or surface-active agents.¹⁰ In these cases, the increase in the leak system judged by the rate of rubidium leak-out from the rabbit lens was demon-

strated while the cation pump mechanism was normal. X-rays and microwave cataracts as observed in rabbits appear to be in vivo examples of compromising the lens permeability properties.^{4, 11}

Sugar cataracts

Another example of lens swelling is that caused by the cataractogenic sugars: glucose, galactose, and xylose. The lens swelling resulting from exposure to galactose, as shown in Fig. 2, is immediate and linear when compared to the ouabain effects. Galactose, like the other two cataractogenic sugars, is converted to sugar alcohol by the enzyme, aldose reductase.¹² The sugar alcohol, because it is not further metabolized effectively and is not able to readily penetrate the lens membranes, once formed in the lens fibers accumulates to high levels. The hypertonicity it creates is immediately corrected by an influx of water.

Of the three cataractogenic sugars the order of decreasing effectiveness in producing changes in lens water and polyol levels is D-xylose, D-galactose, and D-glucose.¹³ This is the same as the order of preference for the sugars by the lens aldose reductase in that the most active substrate for this enzyme is xylose, galactose is next, and glucose is the least active.¹³ In addition, except for the complication in the xylose cataracts, the different rates of cataract

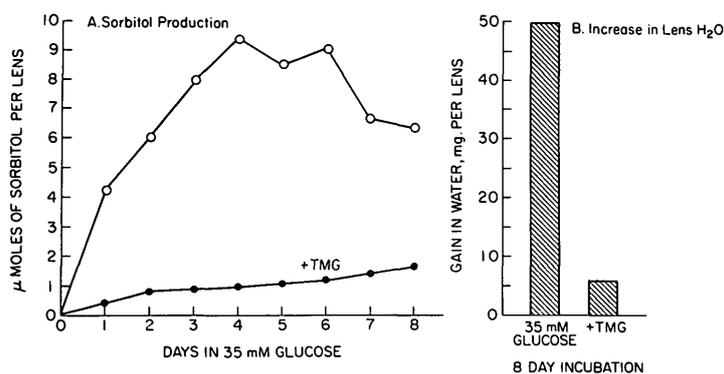


Fig. 5. Tetramethylene glutarate on sorbitol production. Incubation conditions in high glucose medium were described by Chylack and Kinoshita.¹⁶

production in vivo by these sugars are related to the substrate specificity of aldose reductase.¹³ These facts are consistent with the hypothesis that aldose reductase is the primary factor in sugar cataract formation.

The osmotic change that occurs initially with sugar alcohol accumulation does not seriously alter the state of viability. In the early stages of sugar cataract formation the process is reversible. However, when the lens is maintained in a swollen state for a prolonged period, other changes occur, such as the loss of free amino acids and redistribution of cations.¹⁴ Actually, the lens attempts to maintain its normal distribution of cations by accelerating the pump mechanism to compensate for the increase in permeability to cations. Thus, in the galactose-exposed lens the cation flux was shown to dramatically increase in the early stages of galactose cataract.¹⁵ Eventually, however, the increase in cation permeability cannot be compensated and thus Na increases. This sequence of events was illustrated by the incubation of the rabbit lens in 35 mM glucose which simulates conditions in hyperglycemia.¹⁶ As shown in Fig. 4, the initial osmotic change parallels the accumulation of sorbitol. Minimal change in the level of Na occurs during five days of incubation. At this point, there is a sudden increase in Na while the level of sorbitol is beginning to decline. The increase in hydration which initially parallels sorbitol retention is now dependent more on the electrolyte changes.

As details given previously¹ indicate, in the development of a sugar cataract osmotic swelling can be described as occurring in three stages. The first stage, corresponding to the initial vacuolar stage, is one in which the swelling is due almost exclusively to the accumulation of sugar alcohol. The second stage is due both to sugar alcohol and electrolyte increases. In galactose cataract this is the period between the initial vacuolar stage and the appearance of the nuclear opacity. The third stage is when the dense nuclear opacity is observed. At this stage there is a breakdown in the permeability barrier so that electrolytes and sugar alcohol become freely permeable and only the larger proteins are retained. This stage is characterized by a large influx of Na and Cl ions, marked increase in hydration, and a low K level. This is a classical example of Donnan swelling and ultimately occurs when the cation pump fails or when the lens becomes so leaky that the cation pump mechanism cannot keep pace with the rapid influx of Na. At this stage quantities of sugar alcohol found in the cataract are insignificant.

Aldose reductase inhibitors

Since aldose reductase appears to trigger the events that lead to sugar cataract formation, inhibitors of the enzyme were developed to prevent or at least to delay the cataractous process. In Fig. 5, tetramethylene glutaric acid (TMG) was shown to effectively block the accumulation of

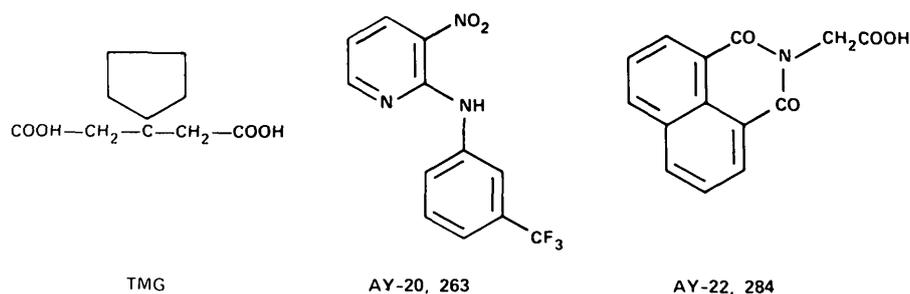


Fig. 6. Aldose reductase inhibitors. TMG = 3,3'tetramethylene glutaric acid. AY-20,263 = α,α,α -trifluoro-N-[2-(3-nitropyridyl)]-m-toluidine. AY-22,284 = 1,3-dioxo-1H-benz(de)-isoquinoline-2(3H)acetic acid.

sorbitol and markedly reduce the increase in lens hydration.¹⁷ When polyol production and the consequent osmotic change were minimized the electrolyte and amino acid changes did not occur.¹⁷ This is further support to the idea that in the development of sugar cataracts, electrolyte and amino acid changes are secondary to the sugar alcohol accumulation and the osmotic change. TMG was shown to be effective in markedly delaying the changes in vitro.^{15, 18} It prevented sugar alcohol formation and osmotic changes that occur in the lenses incubated in medium enriched with either galactose, glucose, or xylose.¹³⁻¹⁷ It also prevented the loss of amino acids, myoinositol, and the changes in electrolyte distribution as well as the morphologic changes that occur in simulating the conditions for sugar cataract development.¹³⁻¹⁷ TMG, however, did not prevent these changes from occurring in rats fed galactose.

The first aldose reductase inhibitor that was effective in vivo was AY-20,263, a compound discovered by Dr. D. Dvornik of Ayerst Laboratories (Fig. 6). This toluidine derivative is yellow in color and has a low aqueous solubility. Advantage was taken of both these properties. A concentrated solution was made up in dimethylsulfoxide (DMSO) which was injected into the vitreous cavity of one of the eyes of a rat fed high galactose. Into the other eye, the same volume of DMSO was injected to serve as a control. As soon as AY-20,263 was injected it precipitated out as the

Table I. Changes in the lenses of rats fed galactose treated with aldose reductase inhibitor, AY-20,263

Galactose level (% of control)	Dulcitol level (% of control)	Lens weight change (% of control)
118 ± 10 (S.D.)	47 ± 12	92 ± 4

Control represents the lens of the untreated eye of a rat fed galactose for five days. The contralateral lens is from the eye injected intravitreally with α,α,α -trifluoro-N-[2-(3-nitropyridyl)]-m-toluidine, AY-20,263. This compound was dissolved in DMSO, 80 mg. per milliliter. Alternate eyes of 24 rats were injected intravitreally with ten microliters of this solution. The eyes serving as controls were injected with the same volume of DMSO without inhibitor. The values given show the per cent differences between the lenses of inhibitor-treated eyes and controls.

DMSO was diluted and the resulting precipitate served as a depot source for the inhibitor. The yellow color also was helpful in indicating when another injection of the inhibitor was necessary. In these experiments, usually no more than two injections were required during the course of galactose cataract development. The results show that administration of AY-20,263 by this manner reduced the level of dulcitol formed and the lens swelling was proportionately less in the treated eye (Table I). In these galactosemic rats the initial vacuoles are usually observed five days after the initiation of the galactose diet. In the treated eye the lens change was delayed by a median value of eight days so that the initial vacuoles occurred on Day 13 (Fig. 7). In the untreated eyes the dense nuclear opacity appears at about 19 days after feeding. In the treated eyes the median delay in the appearance of the nuclear opacity was two weeks. In many

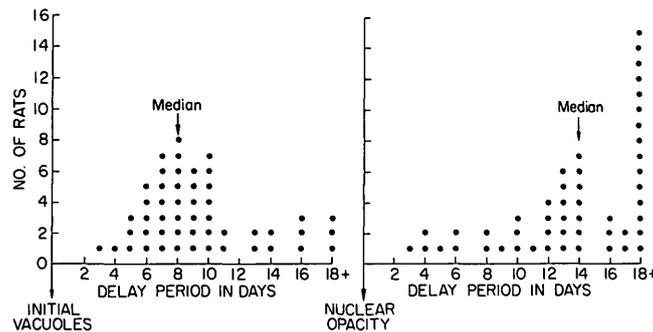


Fig. 7. Delay in the onset of galactose cataract formation by intravitreal injection of AY-20,263. Rats were fed a 50 per cent galactose die. AY-20,263 was injected into the vitreous of one eye of the galactosemic rat.

animals nuclear opacity was not observed even after a delay of 18 days. It is important to note, however, that the opacity eventually develops in all rats. Thus, the action of this aldose reductase inhibitor delays the onset of sugar cataract, but does not prevent it.

The development of AY-22,284 by Ayerst Laboratories is another advance in obtaining a more effective aldose reductase inhibitor (Fig. 6). The actual potency of all three aldose reductase inhibitors is approximately the same in that 10^{-5} M concentrations of these inhibitors cause a 50 per cent inhibition in the lens aldose reductase activity. However, AY-22,284 has a high aqueous solubility and it has low toxicity. In lens culture in high galactose medium it appeared effective in preventing the vacuoles from appearing after three days of incubation and blocking dulcitol accumulation and minimizing lens hydration (Fig. 8). This inhibitor was the first to be effective by oral administration in delaying the onset of cataract formation as judged by the appearance of the dense nuclear cataract.¹⁸ Fig. 9 shows that all the rats fed galactose have developed cataracts by the twenty-ninth day on the galactose diet. However, in those rats fed galactose along with AY-22,284, only 20 per cent developed cataract by 29 days.¹⁸ Oral administration of this aldose reductase inhibitor was thus shown to effectively delay the onset of galactose cataracts.

Dr. Varma, in our laboratory, has been able to show a delay in the galactose cataract formation in rats when the eyes are topically treated with AY-22,284. In the eyes treated with the inhibitor the nuclear opacity was delayed by approximately a week. However, the delivery mechanism must be improved before the topical application can be considered an effective means of controlling this type of cataract.

It is of significance that the three inhibitors of aldose reductase (Fig. 6) are structurally vastly different. However, each has a hydrophobic group and an acidic group. The acidic group in AY-20,263 resides in the aromatic nitro group. The fact that so many compounds inhibit aldose reductase probably is related to the fact that many aldehyde-containing compounds serve as its substrate. It also indicates that other compounds with the necessary structural requirements may turn up as even more potent aldose reductase inhibitors.

Possible clinical implications

The possible clinical use of the aldose reductase inhibitor appears obscure. Even the galactosemic cataracts in infants are of infrequent occurrence. In addition, the galactosemic subjects can be treated simply by withdrawing galactose from the diet. The true diabetic cataracts are also uncommon. Moreover, surveys made thus far seem to indicate that the incidence of cataracts in diabetic subjects is no different

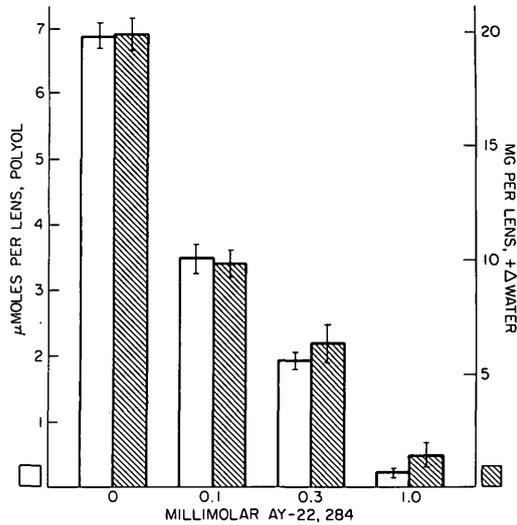


Fig. 8. The effect of AY-22,284 on the galactose-exposed lens. Paired rabbit lenses were used, one lens placed in the control medium and the other in medium containing 30 mM galactose.¹⁷ The increases in sugar alcohol level and water content of lenses incubated for 21 hours in the medium containing galactose with and without AY-22,284 are given as the changes compared to the lens incubated in the control medium. When used, the concentrations of AY-22,284 are given in the figure. The results are corrected to a 175 mg. lens and are given as the mean with the standard deviation of at least five pairs of lenses. The polyol was determined by a gas-liquid chromatography method.¹⁷

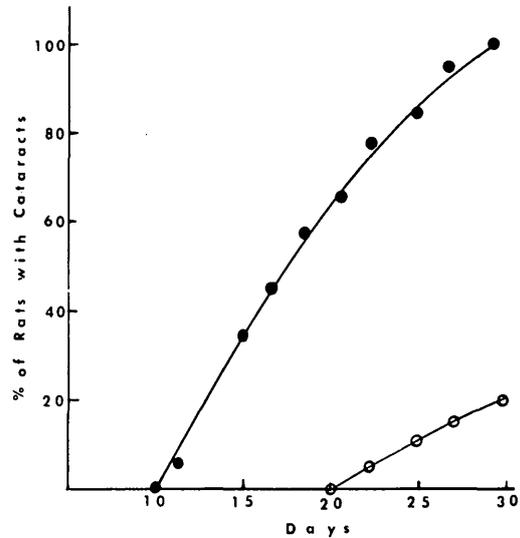


Fig. 9. In vivo effects of AY-22,284 on the rats fed galactose. Conditions are given by Dvornik.⁸ (Dvornik, D., et al.: Polyol accumulation in galactosemic and diabetic rats: Control by an aldose reductase inhibitor. *Science* **182**: 1146-1148, Fig. 1, 1973. Copyright 1973 by the American Association for the Advancement of Science.)

from that in nondiabetic subjects. However, surveys made in England¹⁹ and Germany^{20, 21} indicate that the frequency of cataract extraction is much higher in diabetic than in nondiabetic subjects. In the Oxford study, it was shown that in known female diabetic subjects of the 50- to 70-year-old group, the rate of cataract extraction is nine times higher than in nondiabetic females of the same age group. In known diabetic males of the 50- to 70-year-old group, the frequency of cataract extraction was five times greater than that observed in the nondiabetic group. The criteria for removing cataracts in the diabetic and nondiabetic groups were not different, and the possibility of earlier detection and extraction in the diabetic group has been ruled out. Based on the Oxford

study, it appears that the maturation of cataracts in diabetic subjects occurs much sooner than in nondiabetic subjects.

In experimental animals it has been shown that multiple cataractogenic factors have additive, or even synergistic effects. As illustrated in studies,²²⁻²⁴ when an animal is subjected to two cataractogenic factors, even though they are subliminal, together they appear to have a synergistic effect resulting in formation of a cataract. Dr. Varma has recently observed that cataracts appear much sooner in rats made diabetic and irradiated with x-rays than in either the diabetic or in the x-ray-irradiated rats. Diabetic rats develop a cataract in about three months, while irradiated rats are found with a cataract in 3 to 4 months. When the rats are made diabetic and are also irradiated, cataracts form in approximately a month.

In the middle-aged diabetic subject it is conceivable that the senile process is beginning to become manifested in the lens. If a diabetic component is superimposed on

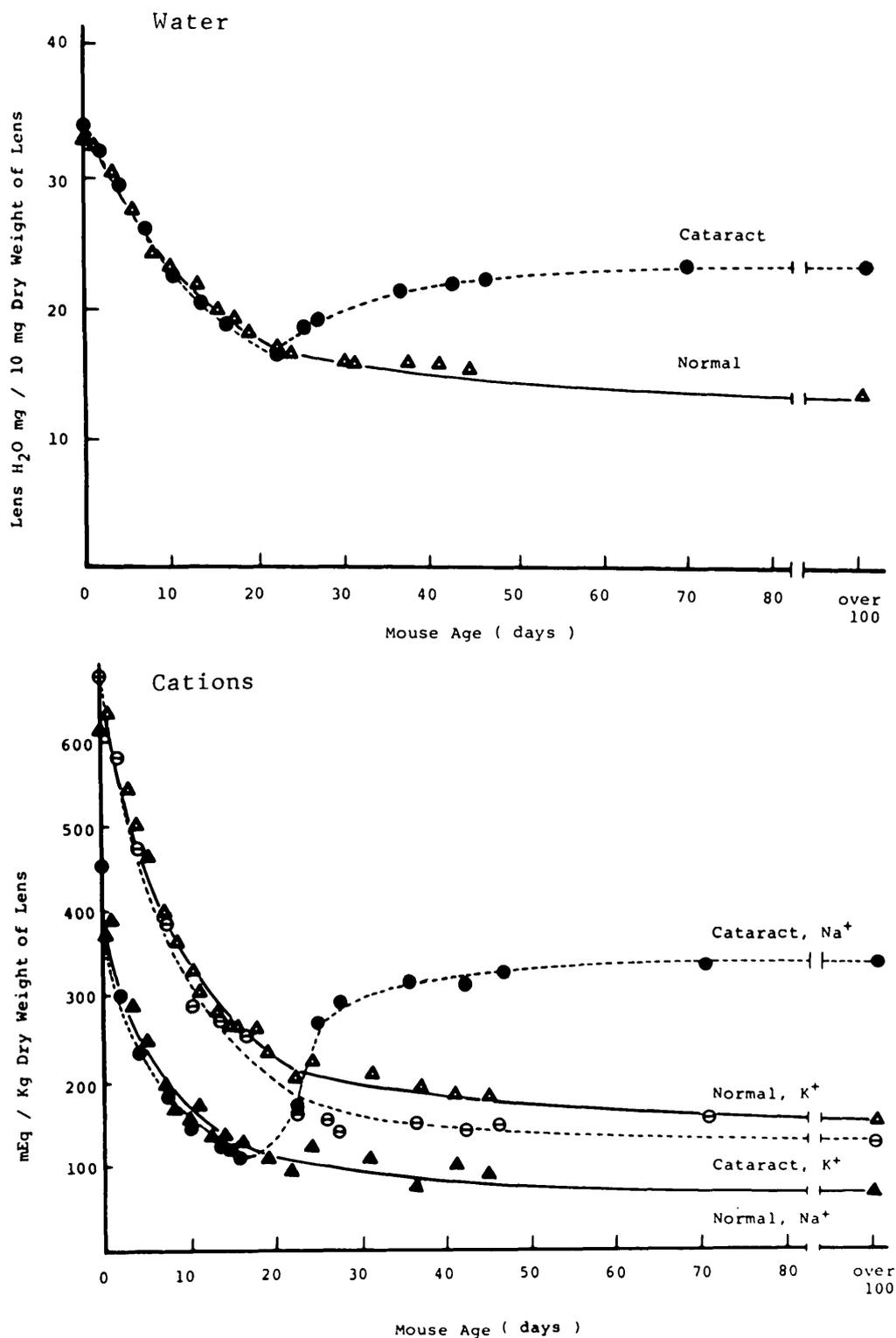


Fig. 10. Electrolyte changes in hereditary mouse cataract.

Table II. Inhibition of Na-K ATPase

Extract (ml.)		Source of lens enzyme	Na-K ATPase (nmole. P/hr.)	Per cent decrease
Normal lens	Cataract			
0	0	Mouse	79	—
0.1	0	Mouse	77	—
0.2	0	Mouse	75	—
0	0.1	Mouse	8	90
0	0.2	Mouse	6	93
0	0	Rat	150	—
0.2	0	Rat	160	—
0	0.1	Rat	74	51
0	0.2	Rat	52	65
0	0	Calf	400	—
0.2	0	Calf	410	—
0	0.1	Calf	240	40
0	0.2	Calf	190	53

Extracts are made from the lenses of cataractous and normal mice in the proportion of two lenses per 1 ml. of Tris buffer, pH 7.0. Only the supernatant fluid is used after centrifugation as the source of inhibitor. Methods used for ATPase assay are essentially those described by Iwata and Kinoshita.³

this situation, the result may be that the two cataractogenic factors together enhance the maturation process of the cataract. If this is the case, the action of an effective aldose reductase inhibitor may be helpful in minimizing the diabetic effects and delaying the cataractous process. A significant delay in the maturation of the cataract would have obvious beneficial effects for the patient.

Hereditary mouse cataract

Another type of cataract involving an osmotic change occurs in a strain of mice that develops a pinhead nuclear opacity three weeks after birth.³ These mice were discovered in Japan and are called the Nakano Cataract Strain. These mice were crossed with the Charles River Strain and a new colony of the Nakano Strain was developed in this country. Except for the cataract, these defective mice seem to be as healthy and grow as well as the control mice. The most obvious change in these cataracts is a sudden increase in lens hydration just about the time the pinhead opacity is observed (Fig. 10). It appears that concomitant with the increase in lens hydration there is also a sudden increase in the lens sodium.³ It does, therefore, appear that the sodium increase is directly related to the osmotic swelling. The elec-

trolyte and osmotic changes occur approximately 20 days after birth, about the time the pinhead opacity is observed. At this stage, the other changes are not remarkable in that slight decreases are observed in potassium, glutathione, and ATP levels. With time, the nuclear opacity becomes more prominent and eventually the opacity covers the entire lens. At this advanced stage a dramatic increase in sodium chloride and loss in potassium are observed, along with marked overhydration, typical of many cataracts, as mentioned previously.

The changes early in the course of the cataract prior to the appearance of the pinhead opacities were examined in hopes of uncovering the initiating factor in this type of cataract. Since the electrolyte disturbance occurs early, the cation pump mechanism in these cataracts was evaluated. As early as 13 days after birth the rubidium ion uptake, which measures the ability of the lens to concentrate potassium ion, is less effective by 50 per cent in these cataracts than in the control mouse lenses.³ The inability of the cataract to concentrate rubidium is substantial and persists throughout the cataractous process. The failure of the cation pump is further manifested in the cataract by loss of effectiveness in extruding Na.³ The defect is observed as early as 13 days. There is a

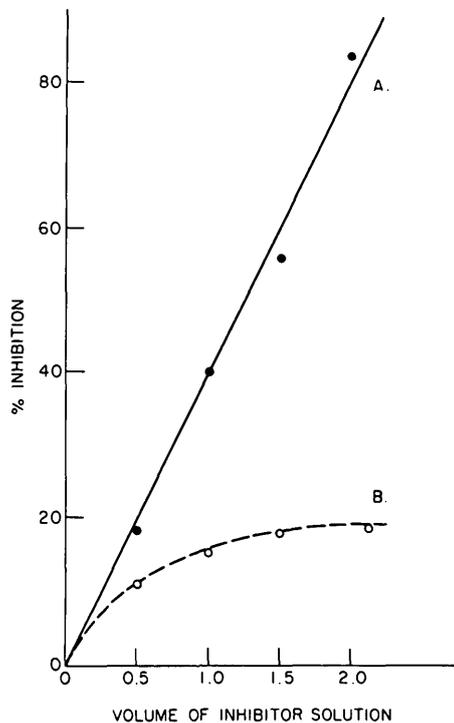


Fig. 11. Action of inhibitor on lens Na-K ATPase. Inhibitor from the mouse cataract extract is incubated for (A) 2 hours (B) 0 hour, before assay for ATPase activity. Methods used as described by Iwata and Kinoshita.³

greater amount of labeled Na taken up by the cataract than by the normal lens.³ The failure to exclude Na becomes more marked as the cataract progresses. This defect in the cation pump mechanism is probably responsible for the gain in Na that is observed in these cataracts. The permeability properties related to cations seem unaffected in the early stages, as evidenced by the fact that the rate of Rb⁸⁶ runout is unchanged. These findings suggested that some mechanism or component of the cation pump mechanism is impaired in these mice. Further examination indicated that the Na-K ATPase is depressed in the lenses of these cataractous mice.³ At 13 days, a 50 per cent decrease in Na-K ATPase activity is observed and the activity is further depressed with age of the mice. It appears quite possible that the factor that initiates the cataract development in

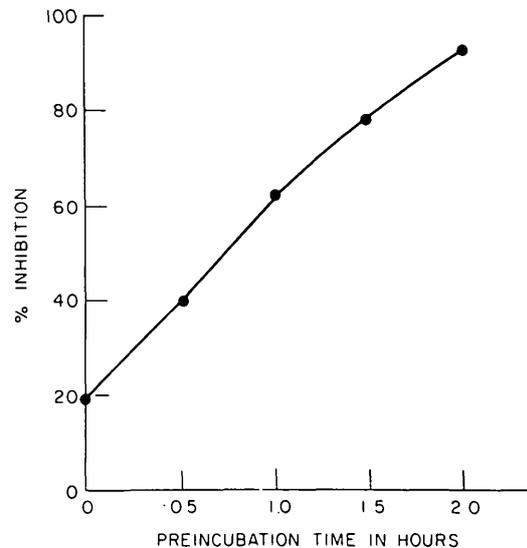


Fig. 12. The effect of pre-incubation time on inhibitory activity.

these mice is a defective lens Na-K ATPase.

Complicating this simple explanation was the fact that in other tissues the Na-K ATPase activity was normal. Thus, an unusual situation seems to exist in these mice in that the genetic defect was expressed only in the lens. This was a little disturbing, so other possible explanations for the manifestation of a hereditary defect were examined. Mr. Merola and I thus explored the possibility that an inhibitor was present in the cataractous mouse lens. In these studies, lenses from mice of about 25 to 30 days old were homogenized in neutral buffer, centrifuged, and the precipitate discarded. The supernatant fluid was tested for the presence of an inhibitor. As a control for this experiment a preparation of lenses from the normal mice of the same age group was taken, and the lens extract was prepared in the same way as that from the cataractous mouse lens. The Na-K ATPase from a normal mouse was used in this study. As shown in Table II, the addition of the cataract extract completely inhibited the mouse lens Na-K ATPase, while the same amount of extract from the normal lens had no effect at all. The cataract extract was also effec-

tive against the Na-K ATPase from rat lens, calf lens, or calf retina. The inhibition was less than that observed for the mouse lens ATPase since the activity of the enzyme was much higher from the other sources.

We decided to use calf lens as the source of Na-K ATPase for all subsequent studies since it could be prepared in large quantities with sufficiently high activity and the enzyme could be stored and was stable in the frozen state. The method used to assay for ATPase activity involved measuring total ATPase activity which is made up of Na-K ATPase and a nonspecific ATPase. The ouabain-sensitive enzyme was taken as the Na-K ATPase. The inhibitor found in the extract of the cataract was effective against Na-K ATPase but had no effect against the nonspecific ATPase.

The inhibitor of Na-K ATPase is nondialyzable and loses inhibitory activity at 100° C. Thus, the inhibitor is a heat-labile, nondialyzable compound.

In the assay of ATPase activity, increasing the inhibitor concentration does not proportionately increase the degree of inhibition. We found that a pre-incubation period is necessary before linearity could be achieved (Fig. 11). After two hours of incubation a linear relation between inhibitor concentration and degree of inhibition is observed. Prolonging the pre-incubation period results in a higher degree of inhibition. As shown in Fig. 12, no plateau is observed because the degree of inhibition continues to rise with time. This is a curious finding which suggests that the association of the inhibitor is a slow process. The interaction of the cataract factor and the Na-K ATPase may better be described as an inactivation rather than an inhibition. Conceivably, the cataract factor may be an enzyme which inactivates the ATPase. It is obvious that further work is necessary before we can begin to understand the complex mechanism. It does appear, however, that in the cataractous mouse lens there is present an inhibitor or inactivator lens Na-K ATPase which is

not present, or at least not detectable with the methods used, in the normal mouse lens. Whether this inactivator is responsible for initiating the cataractous process is an intriguing possibility that requires further study.

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