Mechanism of development of hereditary cataract in mice

Shuzo Iwata and Jin H. Kinoshita

The biochemical evidence regarding a recessively transmitted cataractous trait in mice suggests that an apparent deficiency of Na-K ATPase may be involved in the initiation of this type of cataract. The enzyme defect leads to inefficiency of the cation pump mechanism. This abnormality was demonstrable in 13-day-old mice. At this stage the lens was clear and the electrolyte levels were normal. However, by the twentieth day it became apparent that the defective lens was no longer able to extrude sodium efficiently, therefore sodium content increased. The sudden increase in electrolytes drew water into the lens and an osmotic change occurred. These events preceded the appearance of a "pin-head" nuclear opacity.

Key words: hereditary mouse cataracts, lens Na-K ATPase deficiency, cataracts, lens electrolyte changes, lens cation pump defect.

A strain of mice that develops a lens opacity shortly after birth was first described in Japan by Nakano and associates. This strain was crossed with a Charles River strain of mice so that a colony of mice with a recessively inherited trait leading to the development of cataracts was available for study. The report presented concerns certain biochemical investigations suggesting that a partial deficiency in the lens Na-K adenosine triphosphatase (ATPase) may be responsible for the development of this hereditary cataract.

Materials and methods

The hereditary cataractous strain of mice brought to the Howe Laboratory of Ophthalmology originated from an inbred strain that has been maintained for 12 years in Japan. These mutants were crossed with a strain of Charles River albino mice (Charles River Breeding Laboratory, Wilmington, Massachusetts). By appropriate and selective mating of the first generation of the heterozygous strain, homozygous mice with cataracts were obtained as well as nonmutant animals. The cataract phenotype followed Mendel's laws.

All animals were maintained on the Charles River formula "Bi-Namic Pellet." The animals weighed 1.5 grams at birth and 24 grams at 100 days of age. At designated periods of development, the defective mice and the closely allied nonmutant strains were killed by decapitation. The globes were excised, and the lenses were removed by a posterior approach. They were used to determine chemical changes, and for isotope studies in which the cataracts were compared with nonmutant controls.

Unless otherwise specified, determinations of
Development of hereditary cataract

Fig. 1. Hereditary cataract in mice. A, "Pin-head" nuclear opacity stage. This first sign of opacity occurs on the twenty-third to twenty-fifth postnatal day. B, Mature cataract stage.

Results

Developmental process. The normal and cataractous mice opened their eyes on the fourteenth postnatal day. At the time the eyes opened the lenses of defective mice were still clear. The first sign of opacity occurred 23 to 25 days after birth and appeared as a "pin-head" opacity in the lens nucleus (Fig. 1, A). The area of opacity then slowly enlarged until the entire lens was involved and the mature cataract was formed (Fig. 1, B). The lens ceased to grow when this stage was reached.

These defective mice appeared healthy and grew as well as did the controls. Although a more detailed examination must be performed before concluding that other organs were not affected, it does appear that only the lens is abnormal.

Water content. The changes in lens hy-

Fig. 2. Water content of normal and cataractous lenses. The results are expressed as milligrams of lens water per 10 mg dry weight of lens.

Fig. 3. Cation levels of normal and cataractous lenses. The results are expressed as milliequivatents per kilogram of dry weight of the lens.
Development of hereditary cataract

Dratation of the cataractous mice compared with controls during various stages of development are illustrated in Fig. 2. No difference in lens hydration between the hereditary cataract and control lenses occurred until 22 days after birth. A sudden increase in lens hydration began at this stage. This period occurred a few days prior to the appearance of the "pin-head" opacity. In the developing cataract the lens hydration slowly increased until the cataract matured. After the thirty-fifth postnatal day the increase in hydration was 40 to 70 per cent above that of the normal lens. After birth there was a gradual decrease in water content in the normal lens (Fig. 2).

Sodium and potassium levels. Since electrolytes are an important factor in determining the degree of hydration in tissues in general, the changes in the levels of sodium and potassium during various stages of the cataractous lens were followed. Cation levels in the normal lens paralleled the state of hydration (Fig. 3). After birth the cation levels in the lens gradually decreased with age and reached a plateau after 30 to 40 days. The curves representing cation levels (Fig. 3) are very similar to the curve illustrating the lens hydration changes with age (Fig. 2). In the cataract no difference from the control was observed until after the twentieth day. At this point there was an abrupt increase in total sodium. The sudden elevation in sodium ion coincided with the increase in lens hydration. Over all, the potassium level did not appear to change significantly. This is somewhat misleading in that the results presented are based on dry weight. Actually, the potassium ion concentration did decrease after Day 20, not so much because the absolute level dropped but because of the sudden increase in lens water.

Changes in a number of chemical
parameters occurred in all cataracts. Other properties of the cataract were examined to determine the magnitude of these changes and when they occurred. The results of this survey are summarized in Fig. 4. It is obvious that many changes occurred in the mature cataract. There was a gross distortion in many chemical properties. However, when the mouse lens was examined just prior to the appearance of the "pin-head" opacity, most of the changes were slight except for the increase in sodium. At an earlier stage, 13 days after birth, none of the changes was significant. To obtain some insight into the primary factors involved in the initiation of this type of cataract, other approaches were applied to the 13-day-old mouse lens destined to become a cataract. Studies on the uptake and run out of labeled cations were undertaken for this purpose.

It is generally accepted that the lens transports rubidium in the same manner as it does potassium by the Na-K pump mechanism.\(^7\) Changes in the effectiveness of the pump mechanism were determined by the extent of \(^{86}\text{Rb}\) accumulation in the lens during a particular incubation period. Lenses from the afflicted animals were less able to concentrate \(^{86}\text{Rb}\) than were the controls (Fig. 5). In the 13- to 15-day-old group the effectiveness of accumulating \(^{86}\text{Rb}\) was reduced to 50 to 60 per cent of normal. Thus, at a very early stage of cataract development, a defect in the cation pump mechanism can be demonstrated. Experiments dealing with \(^{24}\text{Na}\) uptake provide additional support of this statement. In this case the pre-cataractous lenses took up more \(^{24}\text{Na}\) than did the controls (Fig. 6). The results show that the defect in the cation pump mechanism is manifested by the inability of the cataracts to exclude sodium as effectively as do normal lenses.

The possibility that these results can be explained by increased permeability to cations was explored next. The rubidium run out experiments indicate (Fig. 7) that the permeability changes are not detectable in the early stages (13 to 15
Fig. 6. Changes in sodium uptake of normal and cataractous lenses. The incubation was for three hours at 37° C. The results are given as the mean ± standard error of at least ten lenses for each period of incubation.

Fig. 8 illustrates the changes in Na-K ATPase activity in the early stage of cataract development. To obtain these results, determinations were made on lenses of approximately 50 defective mice and on a similar number of controls. At the early stage (13 to 15 days) the lens weight of cataract was no different from that of the control lens; therefore the results are presented on a per lens basis. There was an approximately 50 per cent drop in Na-K ATPase activity in the precataractous lenses compared to the controls (Fig. 8). This magnitude of decrease in lens enzyme activity was consistently observed in the 13- to 15-day-old defective mice. Thus it appears probable that a depressed Na-K ATPase level accounted for the defect in the cation pump mechanism.

An investigation of the heterozygous mice that do not develop cataracts revealed that their lenses contained normal levels of Na-K ATPase and that they were able to concentrate rubidium to the same extent as did the nonmutant controls.

Discussion

The cataractous mice studied were derived from a strain discovered by Nakano and associates. In these mice the defective trait is transmitted recessively. These mice are thus distinguished from the mouse
strain that develops a cataract known to be caused by dominant mutation. The mutant carrying the dominant gene is known as "Cararact Fraser" (CatFr). To be consistent and to make a distinction from the CatFr, the recessively inherited mutant will be designated as "Cataract Nakano" (CatNa). In addition to the difference in the mode of transmission of the inherited traits, there is also a difference in the morphology of these two forms of cataracts. CatFr mice are described as having anterior pole cataracts, while CatNa mice are found to develop a "pin-head" nuclear opacity. Brown and associates showed that microscopic changes appear in the CatNa lens on the fourteenth day. These changes consist of the appearance of abnormal cortical lens fibers that are swollen, granular, and, at times, appear to liquefy. In the CatFr mice the microscopic changes are detectable in embryonic life and appear as cytoplasmic degeneration. The epithelium is not involved until after birth, at which time the epithelium proliferates and becomes atypical. Extensive microscopic studies of changes in the lens epithelium and capsule of the CatNa mice thus far have not been described.

Most of the chemical properties of the lens appear normal in the 13-day-old CatNa mice. Major changes in electrolytes are not observed until the mice are at least 20 days old. However, in the 13-day-old CatNa mice certain dynamic aspects of the lens cation pump mechanism can be shown to be abnormal. The defective gene is expressed by a partial deficiency—a 50 per cent reduction—of the lens Na-K ATPase activity. This apparent deficiency in Na-K ATPase activity produces a proportional decrease in the effectiveness of the cation pump. We have some evidence that enzyme deficiency is observed as early as 7 days of age, but whether or not it occurs at birth has been technically too difficult to establish. Apparently the lens is able to maintain electrolytes and hydration at normal values with a partially defective cation pump mechanism until the age of 20 days.

Instead of a deficiency of enzyme, the
Defective gene may be expressed by the presence of an inhibitor. This possibility is being explored, but thus far we have been unable to demonstrate such an inhibitor. It may be somewhat surprising that this hereditary disease affects only the lens. Except for the cataract there appears to be no other abnormalities. Our preliminary studies reveal that these defective mice appear to have normal levels of Na-K ATPase in the kidney and brain. Thus it appears that Na-K ATPase is affected only in the lens. One would have thought that to explain the partial deficiency of the ATPase, the affected gene would lead to decrease enzyme synthesis or that the enzyme synthesized would be slightly altered. If this were the case other tissues would be affected as well, especially since the enzyme is so widely distributed. The fact that only the lens seems to be involved suggests that the expression of the gene defect is more complicated. It is interesting that a more extensively studied hereditary disorder, glucose-6-phosphate (G-6-PD) dehydrogenase deficiency, also has complicated manifestations.\(^\text{17}\) In the red cell the defect results in partial lowering of G-6-PD activity. However, the leukocyte G-6-PD activity was found to be normal in Negroes. In other tissues which are affected, the degree of decrease in enzyme activity is always much less than that observed in the red cell.

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