(*23*). Since the hormonally defined medium we used was designed for a continuous cell line, it is quite possible that our isolated "normal" cells may require additional hormones or factors (or both) to achieve a maximal growth rate. In summary, we have demonstrated that it is now possible to isolate and maintain a single kidney cell type that exhibits transport properties in a totally defined system.

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14. The hormonally defined medium, originally defined for the Madin-Darby canine kidney line (MDCK) (*15*), contains: insulin (5 μg/ml), prostaglandin $E_1$ (25 ng/ml), hydrocortisone ($5 \times 10^{-8}M$), triiodothyronine ($5 \times 10^{-12}M$), transferrin (5 μg/ml), and sodium selenite ($10^{-8}M$) in a mixture (1:1) of Ham's F-12 and Dulbecco's modified Eagle's medium. Maintenance medium consists of: a mixture (1:1) of Ham's F-12 and Dulbecco's modified Eagle's medium and fetal calf serum (5 percent by volume). Both media contained *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (10 m*M*, *p*H 7.4), sodium bicarbonate $NaHCO_3$ (1.1 mg/ml), penicillin (50 unit/ml), and streptomycin (50 μg/ml). The media, serums, and antibiotics were obtained from Gibco. All other reagents were purchased from Sigma.
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## Persistence of Crystallin Messenger RNA's with Reduced Translation in Hereditary Cataracts in Mice

Abstract. *In vitro translation experiments showed that the lens fiber cells of two hereditary cataracts in mice (Nakano and Philly) possessed a full complement of crystallin messenger RNA's, despite severely reduced synthesis of crystallin in these cells. The reduction in synthesis in the lens fiber cells correlated with the increase in $Na^+$ and the decrease in $K^+$, which occurs during cataractogenesis. In contrast to the fiber cells, the epithelial cells continued to synthesize crystallins in the cataractous lenses. Crystallin synthesis was stimulated in the fiber cells by raising the $K^+$ concentration and lowering the $Na^+$ concentration in the cultured lenses. The reduction in crystallin synthesis in the initial stages of cataractogenesis in the Nakano and Philly lenses thus appears to be due to poor utilization of crystallin messenger RNA's in the fiber cells.*

Cataracts are lens opacities that seriously affect vision and may lead to blindness. One important initiating factor of cataractogenesis appears to be lens hydration, which is associated with a large increase in the intralenticular concentration of $Na^+$ and a decrease in the concentration of $K^+$ (*1*). Examples of osmotic cataracts with an elevated $Na^+/K^+$ ratio include galactose cataracts in rats (*2*) and hereditary cataracts in Nakano (*3*) and Philly (*4*) mice. Experiments with cultured embryonic chicken lenses have shown that an increase in the $Na^+/K^+$ ratio, comparable to that occurring in osmotic cataracts, is correlated with a differential reduction in crystallin synthesis (*5*), suggesting that crystallin synthesis may also be affected in mammalian cataracts associated with cation imbalances (*6*). This possibility was supported by experiments correlating a differential reduction in crystallin synthesis with alterations in the intralenticular concentration of $Na^+$ and $K^+$ in ouabain-treated normal (*7*), Nakano (*7*), and Philly (*8*) mouse lenses as well as in galactosemic (*9*) and hypocholesteremic (*10*) rat lenses. Differential reductions in protein synthesis have also been observed in human lenses cultured in the presence of ouabain (*11*). We now report that changes in $Na^+$ and $K^+$ affect crystallin synthesis by interfering with the efficient utilization of crystallin messenger RNA's (mRNA's) in mammalian cataracts.

Protein synthesis was examined in cultured lenses from 30-day-old normal (NIH) and Philly mice and from 40-day-old Nakano mice. By 30 days after

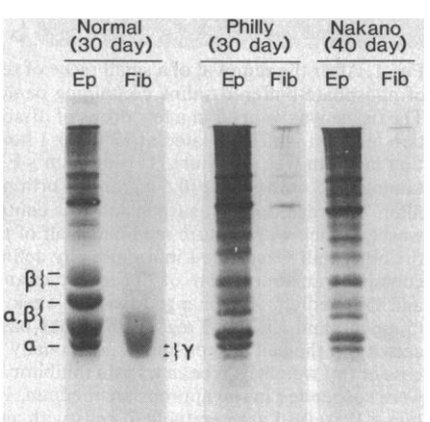


Fig. 1. Autoradiograms of sodium dodecyl sulfate (SDS)-urea-polyacrylamide gels of lens proteins from normal, Philly, and Nakano lenses. Three lenses were removed from the eyes and incubated in 0.5 ml of Ham's F-10 medium (*12*) supplemented with fructose (8.48 g/liter) and [$^{35}$S]methionine (New England Nuclear; 1 mCi/ml; 800 Ci/mmole) for 8 hours at 37°C in a humidified atmosphere of 5 percent $CO_2$ and 95 percent air. The epithelial cells were separated from the fiber cells under the dissecting microscope. They were dissolved in 0.75 ml of sample buffer (2 percent SDS, 8*M* urea, 1 percent 2-mercaptoethanol, 10 percent glycerol, 0.1*M* tris, *p*H 6.8) and the fiber cells were dissolved in 1.5 ml of the same buffer; 15 μl of the fiber cell homogenate and 30 μl of epithelial cell homogenate were subjected to electrophoresis in a 12 percent polyacrylamide gel slab and to autoradiography (*13*). The designation of the bands as α-, β-, or γ-crystallin was established by immunoprecipitation (*8*). The broad upper band, identified as γ-crystallin in the fiber cells, also contained α- and β-crystallin polypeptides.


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birth, the Philly lens has an anterior cortical subcapsular opacity (*4*) and by 40 days the Nakano lens has a pronounced nuclear opacity (*3*). Batches of three lenses were incubated with [$^{35}$S]methionine in Ham's F-10 medium (*12*). After 8 hours, the epithelial cells were surgically separated from the fiber cells and the proteins were fractionated by electrophoresis (*13*). The autoradiograms shown in Fig. 1 demonstrate that there was a considerable amount of [$^{35}$S]methionine incorporated into the proteins of the normal and cataractous lens epithelial cells, although incorporation into the crystallins was differentially reduced in the epithelial cells of the Philly and Nakano lenses. Even larger differences were observed between the fiber cells of the normal and cataractous lenses. In contrast to the fiber cells of the normal lens, extremely little [$^{35}$S]methionine was incorporated into the crystallins of the fiber cells of the Philly or Nakano lenses. Immunoprecipitation tests indicated that the fiber cells of the normal lens synthesized the $\alpha$, $\beta$-, and $\gamma$-crystallins (data not shown) (*8*). The reduction of [$^{35}$S]methionine incorporation into the crystallins of the fiber cells was already evident by 20 days in the Philly lens and by 30 days in the Nakano lens; these times correlate well with the times of the elevation of the $Na^+/K^+$ ratio in the Philly (*4*) and Nakano (*3*) lenses. The incorporation of [$^{35}$S]methionine into the higher molecular weight noncrystallin polypeptides of the fiber cell preparations from the cataractous lenses was probably due mostly to contamination by residual epithelial cells.

In order to determine whether the reduced [$^{35}$S]methionine incorporation into the crystallins of the fiber cells of the cataractous lenses was due to the loss of crystallin mRNA's, we translated isolated RNA's from the fiber cells of Philly and Nakano lenses in a rabbit reticulocyte cell-free system. The autoradiograms shown in Fig. 2 reveal, surprisingly, that the fiber cells of the 30-day-old Philly and the 51-day-old Nakano lenses contained a full complement of crystallin mRNA's. The crystallins made on the mRNA's from the cataractous lenses comigrated with those made on the mRNA's from normal lenses. Separate experiments showed that the $\alpha$-, $\beta$-, and $\gamma$-crystallins synthesized in the reticulocyte lysate immunoprecipitated with the appropriate antiserum (data not shown).

We next attempted to stimulate crystallin synthesis in the fiber cells of the

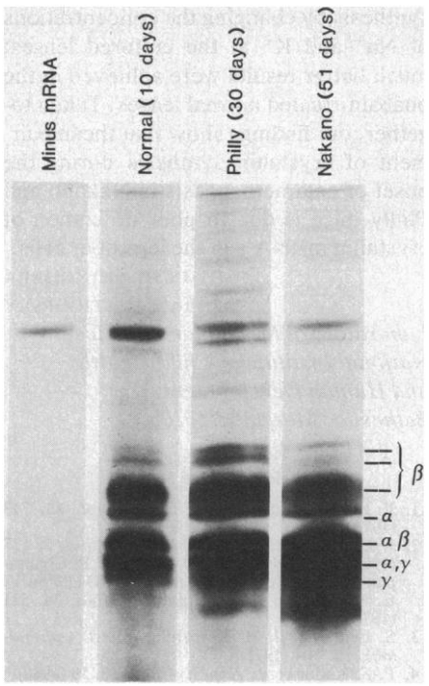


Fig. 2. Autoradiograms of SDS-urea-polyacrylamide gels of proteins derived by translation of total RNA's from fiber cells of normal, Philly, and Nakano lenses. The RNA's were extracted with a mixture of phenol, chloroform, and isoamyl alcohol (50:50:1) (*16*), precipitated with ethanol, dissolved in water, and "translated" in a rabbit reticulocyte lysate kit (New England Nuclear). The autoradiograms represent translation products of RNA from the fiber masses of two lenses.

cultured 30-day-old Philly and the cultured 40-day-old Nakano mouse lenses since they contain the crystallin mRNA's. The lenses were precultured for 18 hours in medium enriched with $Na^+$ or with $K^+$ (see legend to Fig. 3). The intralenticular concentrations of $Na^+$ and $K^+$ approached those of the medium in the cataractous lenses after the 18 hours of culture, as judged by atomic absorption spectroscopy for $Na^+$ and by flame photometry for $K^+$. The intralenticular $Na^+$ and $K^+$ concentrations equilibrate with their external concentrations since the Nakano (*1*, *3*) and Philly (*4*) lenses are permeable to these ions. The precultured lenses were incubated with [$^{35}$S]methionine for an additional 8 hours, and the proteins from the fiber cells were examined by electrophoresis and autoradiography. The scans in Fig. 3 show that incorporation of [$^{35}$S]methionine into the crystallins occurred only in the lenses containing a high concentration of $K^+$ and a low concentration of $Na^+$. Incorporation of [$^{35}$S]-methionine into the crystallins was also stimulated by increasing the $K^+$ and reducing the $Na^+$ concentrations in the lens fiber cells of 51-day-old Nakano mice. In other experiments, stimulation of [$^{35}$S]methionine incorporation into the crystallins occurred in the fiber cells of Nakano and Philly mouse lenses by increasing the $K^+$ concentration above 30 m*M* even when the $Na^+$ concentration remained at 100 m*M*. Either potassium

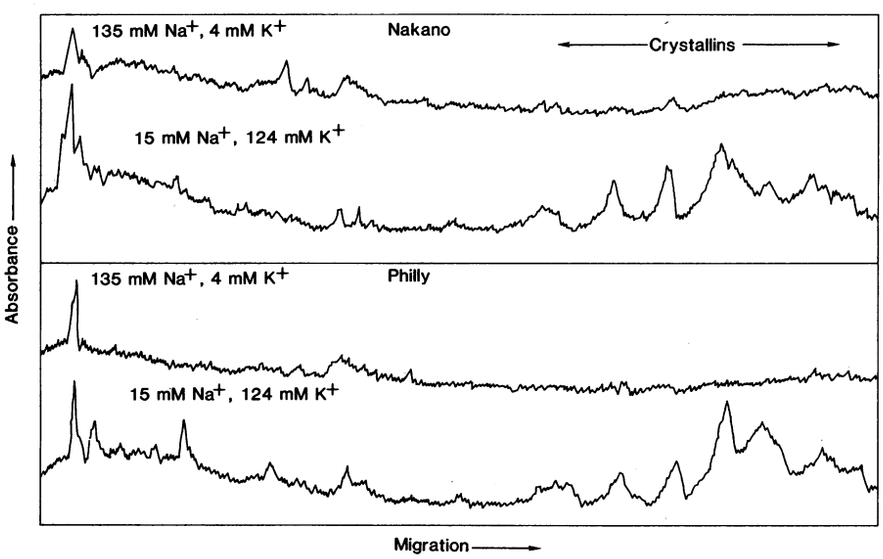


Fig. 3. Scans of autoradiograms of SDS-urea-polyacrylamide gels of [$^{35}$S]methionine-labeled proteins from the fiber cells of 40-day-old Nakano and 30-day-old Philly lenses cultured in medium containing different concentrations of $Na^+$ and $K^+$. Three lenses were cultured either in Ham's F-10 medium containing, as usual (*12*), 120 m*M* NaCl or in modified medium in which the NaCl was replaced with 120 m*M* potassium acetate. Since Ham's F-10 medium contains an additional 15 m*M* $Na^+$ and 4 m*M* $K^+$ from other salts, the final concentration of these cations is as given in the figure. The Ham's F-10 medium was always supplemented with fructose (8.48 g/liter).

acetate or potassium chloride was able to promote [$^{35}$S]methionine incorporation into cataractous lenses, indicating the importance of the cation in this process. Optimum incorporation of [$^{35}$S]methionine into the crystallins was observed between 30 m*M* $K^+$ (90 m*M* $Na^+$) and 60 m*M* $K^+$ (60 m*M* $Na^+$). At best [$^{35}$S]methionine incorporation into the crystallins of the cataractous lens fiber cells was approximately 30 percent of the incorporation in the normal lens fiber cells. The failure to obtain greater incorporation of [$^{35}$S]methionine into the crystallins may be due to decreased amounts of adenosine triphosphate (ATP) or to reduced specific activities of the intracellular pool of [$^{35}$S]methionine in the Philly (*4*, *8*) and Nakano (*3*, *7*) lenses. The differences in [$^{35}$S]methionine incorporation could not be accounted for by differences in uptake of the labeled amino acid.

Further evidence indicating the importance of the intracellular cation environment for optimal crystallin synthesis was obtained in ouabain-treated lenses from 13-day-old normal mice. Ouabain is an inhibitor of $Na^+$,$K^+$-adenosine triphosphatase (*14*) and increases the $Na^+/K^+$ ratio in cultured lenses (*15*). As reported previously (*7*), ouabain treatment ($2 \times 10^{-3}$ *M*) reduced crystallin synthesis at least 95 percent in the cultured lenses. Approximately 80 percent recovery of crystallin synthesis was obtained when the $K^+$ concentration was increased to 50 m*M* and the $Na^+$ concentration was decreased to 89 m*M* in the lenses cultured in the presence of ouabain (data not shown).

The above experiments indicate that the reduction in crystallin synthesis during the early phases of cataractogenesis in the Philly and the Nakano lenses occurs principally in the fiber cells and is due to underutilization rather than degradation of crystallin mRNA's. We do not know whether or not the crystallin mRNA's in the fiber cells are confined to the clear regions of the cataractous lenses or for how long the crystallin mRNA's remain in the Nakano or Philly lens. The fact that changes in the intralenticular concentrations of $Na^+$ and $K^+$ may stimulate crystallin synthesis in the cultured lens of the Nakano or Philly mouse or in the ouabain-treated lens of the normal mouse supports the idea that alterations in the concentration of these cations contribute to the reduction in crystallin synthesis in osmotic cataracts (*5*, *6*). It is likely that other factors besides ions also contribute to the limitation of crystallin synthesis in the Nakano and Philly lens since we were only able to obtain partial recovery of crystallin synthesis by changing the concentrations of $Na^+$ and $K^+$ in the cultured lenses; much better results were achieved in the ouabain-treated normal lenses. Taken together, our findings show that the impairment of crystallin synthesis during the onset of cataractogenesis in Nakano and Philly mice is due to poor utilization of crystallin mRNA's in the lens fiber cells.

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## Dorsal Root Ganglion Neurons Are Destroyed by Exposure in utero to Maternal Antibody to Nerve Growth Factor

Abstract. *Rats and guinea pigs, when immunized with mouse nerve growth factor, produce antibodies that cross-react with their own nerve growth factor. The antibodies reach developing offspring of these animals both prenatally (rats and guinea pigs) and postnatally (rats). Depriving the fetus of nerve growth factor in this way results in the destruction of up to 85 percent of dorsal root ganglion neurons as well as destruction of sympathetic neurons. Sensory neurons of placodal origin in the nodose ganglion were not affected. These data demonstrate that dorsal root ganglion neurons go through a phase of nerve growth factor dependence in vivo.*

Nerve growth factor (NGF) is required for maintenance and survival of sympathetic neurons. The most compelling evidence for this is that heterologous antibodies to NGF, when passively transferred to newborn animals, destroy sympathetic neurons [immunosympathectomy (*1*)]. It has also been shown that NGF is required for the survival of sympathetic neurons in vitro. Similarly, NGF enhances the survival in vitro of dorsal root ganglion (DRG) neurons taken from embryonic chicks (*2*), embryonic rodents (*3*), newborn rodents (*4*), and human embryos (*5*). However, antibodies to NGF, when administered to newborn rodents, do not destroy sensory neurons (*1*). Hence, a physiological role for NGF in the development of sensory neurons in vivo is not yet established. In a recent study in which we used an experimental autoimmune approach, we found that there may be a permanent effect on dorsal root ganglia in rats exposed in utero to maternal antibodies to mouse NGF (*6*). We now report that rats and guinea pigs immunized with mouse NGF produce antibodies that cross-react with their own NGF. Antibodies to NGF, transferred to the offspring in utero, caused massive destruction of both peripheral sympathetic neurons and DRG sensory neurons. In the rat, exposure to maternal antibodies to NGF postnatally in milk destroyed sympathetic neurons, but not dorsal root ganglion neurons.

Adult female Sprague-Dawley rats and outbred guinea pigs were immunized with mouse NGF (*7*). These two species were chosen for study because they represent species in which antibody is passively transferred to offspring at different times during development. The rat receives small amounts of antibody prenatally and greater quantities postnatally via the milk, whereas the guinea pig receives large amounts of maternal antibody prenatally (*8*). For initial immunizations, 100 $\mu$g (rats) or 200 $\mu$g (guinea


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