

REMOVAL OF CORNEAL CRYSTALS BY TOPICAL CYSTEAMINE IN NEPHROPATHIC CYSTINOSIS

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Abstract In patients with nephropathic cystinosis, corneal crystals develop by one year of age; they progressively accumulate and eventually cause recurrent corneal erosions and photophobia. After an *in vitro* study of cystinotic corneal stromal cells showed cystine depletion by cysteamine and after topical cysteamine was determined to be nontoxic in rabbits, we performed a controlled double-blind clinical trial of 10 mM cysteamine eyedrops in young patients with cystinosis, using one eye for treatment

and the other as the control. Two children begun on the protocol before two years of age had a striking decrease in the number of corneal crystals in the cysteamine-treated eye within four to five months of entering the study.

Cysteamine eyedrops appear to be safe and efficacious in the short-term treatment of patients with cystinosis who are under two years of age. The long-term value of such treatment and its effectiveness in older patients remain to be determined. (*N Engl J Med* 1987; 316:775-9.)

IN nephropathic cystinosis, a rare lysosomal storage disease, cystine accumulates within cells to levels 50 to 100 times normal.¹⁻³ The basic defect of this autosomal recessively inherited disorder is an impairment of normal carrier-mediated transport of cystine across the lysosomal membrane⁴⁻⁷; obligate heterozygotes for the cystinosis gene have half the normal amount of lysosomal cystine transport.⁸ In cystinotic cells, free nonprotein cystine, which is poorly soluble in an aqueous solution, reaches such high concentrations that it forms crystals in many tissues, including the kidneys, bone marrow, intestine, liver, spleen, pancreas, thyroid, lymph nodes, retinal pigment epithelium,⁹ conjunctiva, and corneas.¹⁻³

Intralyosomal cystine accumulation in cystinosis apparently interferes with the function of different organs at different rates, resulting in a characteristic sequence of clinical manifestations. Patients are normal at birth, but renal tubular Fanconi's syndrome develops between 6 and 18 months of age, with failure to reabsorb water, electrolytes, bicarbonate, glucose, phosphate, calcium, magnesium, amino acids, carnitine,¹⁰ and other small molecules. Consequently, infants often present with acidosis, dehydration, electrolyte imbalance, failure to grow, or hypophosphatemic rickets. Although the growth failure persists unless specific cystine-depleting therapy is given, the fluid, electrolyte, and nutrient deficiencies can be corrected by oral replacement therapy. This treatment, however, has no effect on the renal glomerular deterioration that begins in the first years of life and progresses inexorably to end-stage kidney disease. Uremia generally occurs by 10 years of age,¹¹ requiring dialysis or renal transplantation.¹² In addition to renal damage, frequent complications of cystinosis include hypothyroidism¹³ and hypohidrosis¹⁴ in young patients and visual impairment,⁹ diabetes mellitus, and neurologic deficits among older patients who have undergone renal transplantation.¹⁵

In the cornea, cystine crystals generally appear by one year of age³ and are pathognomonic of cystinosis.

The corneal crystal accumulation progresses with age and is considered partly responsible for the photophobia, recurrent corneal erosions, and secondary blepharospasm that complicate longstanding cystinosis.⁹

In addition to replacement of renal losses, the therapy of nephropathic cystinosis now includes oral administration of cysteamine (β -mercaptoethylamine), a free thiol with a marked cystine-depleting ability.¹⁶ Cysteamine has been shown to stabilize renal glomerular function and to improve growth in children with cystinosis.¹⁷ However, no retardation in the rate of corneal crystal accumulation has been attributed to systemic cysteamine after as long as eight years of therapy.

We determined that cysteamine effectively reduced the cystine content of cystinotic corneal cells in culture. A double-blind, placebo-controlled trial of topical cysteamine eyedrops was then initiated to study the effects on corneal crystal formation in two young children (less than two years of age) with nephropathic cystinosis.

METHODS

Tissue Culture

A penetrating keratoplasty was performed on a 13-year-old boy with nephropathic cystinosis to achieve partial relief of incapacitating photophobia and blepharospasm caused by recurrent corneal erosions.¹⁸ The corneal stromal cells cultured from the keratoplasty specimen were grown to confluence in Eagle's minimal essential medium (GIBCO, Grand Island, N.Y.) containing 10 percent fetal-calf serum.¹⁹ Cultures (25 cm²) were exposed to 0 to 1 mM cysteamine (Sigma, St. Louis) for up to two hours at 37°C. The cells were harvested by trypsinization, washed three times with phosphate-buffered saline, and assayed for cystine with the cystine-binding protein method²⁰ and for protein with the method of Lowry et al.²¹

Culture plates were examined frequently by phase-contrast microscopy during this experiment. Several plates were fixed in glutaraldehyde and examined by electron microscopy.

Studies in Animals

Cysteamine eyedrops, 0.11 percent (10 mM) or 0.55 percent (50 mM), were prepared under sterile conditions in normal saline solution without preservatives and were placed in one eye in eight albino rabbits every hour, eight hours daily, for 21 days. Normal saline drops were placed in the other eye as part of the randomized and blinded trial. The animals were examined daily for conjunctival injection and other adverse ophthalmologic effects. At the end of the trial, the rabbits were killed and routine histologic examination of the ocular tissues was performed with a light microscope.

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Topical-Cysteamine Protocol

Permission to use topical cysteamine was obtained by amending the Food and Drug Administration's Investigational New Drug Exemption no. 11065 (Dr. J.G. Thoene, University of Michigan). After approval by the National Eye Institute institutional review board, a double-blind, randomized trial of 0.11 percent (10 mM) cysteamine eyedrops in normal saline, as compared with normal saline alone, was initiated. One drop of cysteamine in normal saline was placed in one eye, randomly selected, hourly while the subject was awake. Normal saline alone was placed in the other eye according to the same schedule. Two young children with nephropathic cystinosis, whose corneas had not yet become packed with cystine but did contain visible crystals on slit-lamp biomicroscopy, were enrolled in the study. An ophthalmic nurse taught their mothers how to maintain sterility in administering the eyedrops and how to maintain records of each application on a form supplied to them. Fresh bottles of eyedrops were used every five days to reduce the risk of contamination.

The ocular evaluation included slit-lamp biomicroscopy and photography, which was extremely difficult because of the variable and inconsistent cooperation of the very young children. A Zeiss photo-slit lamp with stereoscopic accessories, beam splitters, twin camera bodies, and side-arm adapters was employed. Direct focal illumination with a moderately wide slit beam (5 mm), an aperture setting at f/22 or f/32, a flash-intensity setting of 2, Ektachrome 200 film, and optimal magnification (25 \times) was used except where specifically noted.

Patients

Patient 1 was a white boy first seen at the National Institutes of Health at the age of 14 months. The diagnosis of nephropathic cystinosis was confirmed by a leukocyte cystine level of 10 nmol of half-cystine per milligram of protein (normal, ≤ 0.2). Oral cysteamine therapy (60 mg per kilogram of body weight per day) subsequently maintained leukocyte cystine levels (measured five hours after a dose) below 1 nmol of half-cystine per milligram of protein. On initial ophthalmic examination at 14 months of age, sparse corneal crystals were evident on stereophotography.

Patient 2 was a white girl first seen at the National Institutes of Health at the age of 13 months. The diagnosis of nephropathic cystinosis was made at 11 months of age on the basis of a leukocyte cystine level of 6.9 nmol of half-cystine per milligram of protein. Treatment with oral cysteamine was begun at 13 months of age, and by 15 months the patient was receiving approximately 70 mg of cysteamine per kilogram per day and the leukocyte cystine level was approximately 1 nmol of half-cystine per milligram of protein. Slit-lamp biomicroscopical examination at the age of 13 months revealed bilateral and symmetrical corneal crystal deposition.

RESULTS

In Vitro Studies

The nonprotein cystine content of the cells ranged from 11.0 to 38.7 nmol of half-cystine per milligram of protein in three different cultures, as compared with a published value of 0.98 nmol of half-cystine per milligram of protein in cultured corneal stromal cells from an adult patient with cystinosis.²² The normal control value in the present study was 0.27 nmol of half-cystine per milligram of protein, and published normal values are less than 0.4.²² When exposed to 1 mM cysteamine, the cystinotic corneal cells lost 82 percent of their cystine within 30 minutes and 96 percent within two hours (Fig. 1). Exposure to 0.1 mM cysteamine for one hour depleted the cells of 76 percent of their initial cystine content (data not shown). Corneal stromal cells, obtained from the keratoplasty specimen and maintained in tissue culture, did not differ greatly

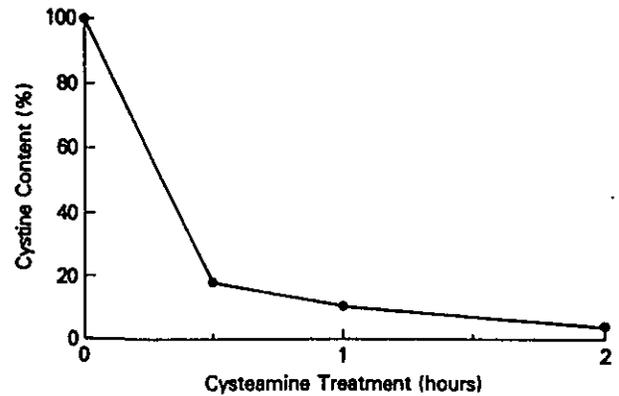


Figure 1. Cystine Depletion by Cysteamine in Corneal Stromal Cells from a Patient with Nephropathic Cystinosis.

Confluent cells were exposed to 1 mM cysteamine for the times indicated and were then harvested and assayed for cystine. The initial cystine content was 19.9 nmol of half-cystine per milligram of protein. Results are means of duplicate determinations.

from normal cells on examination by either light or electron microscopy. The cells contained regular microorganelles and numerous inclusion bodies. There were no specific morphologic alterations attributable to cysteamine exposure.

Studies in Animals

Daily clinical evaluation and a complete histopathological examination of the rabbits' ocular tissues were performed after 21 days of eyedrop administration in eight animals. No differences between cysteamine-treated and normal saline-treated eyes were observed (data not shown).

In Vivo Studies

In Patient 1, sparse corneal crystals present at 14 months were difficult to observe because of poor cooperation but were documented photographically in the one eye (the right) that could be photographed. By 18 months, however, crystals were evident on slit-lamp biomicroscopy as well as photography and were present in similar amounts in both eyes. The crystals remained in both eyes until the patient was enrolled in the cysteamine-eyedrop protocol at 21 months of age (Fig. 2a). After one month of therapy, there was a clinical impression of a decrease in the number of crystals in the right eye. After three months, the difference between the two eyes was evident, and by five months, the right central cornea appeared to be free of crystals, whereas the crystal deposition in the left cornea was unchanged (Fig. 2b). These findings were documented photographically and confirmed on slit-lamp biomicroscopy by three independent ophthalmologists who were unaware of the identity of the cysteamine-treated eye and had not consulted before their evaluations.

After five months of therapy, because of the marked discrepancy in the crystal content between the two eyes, it was considered inappropriate to continue the

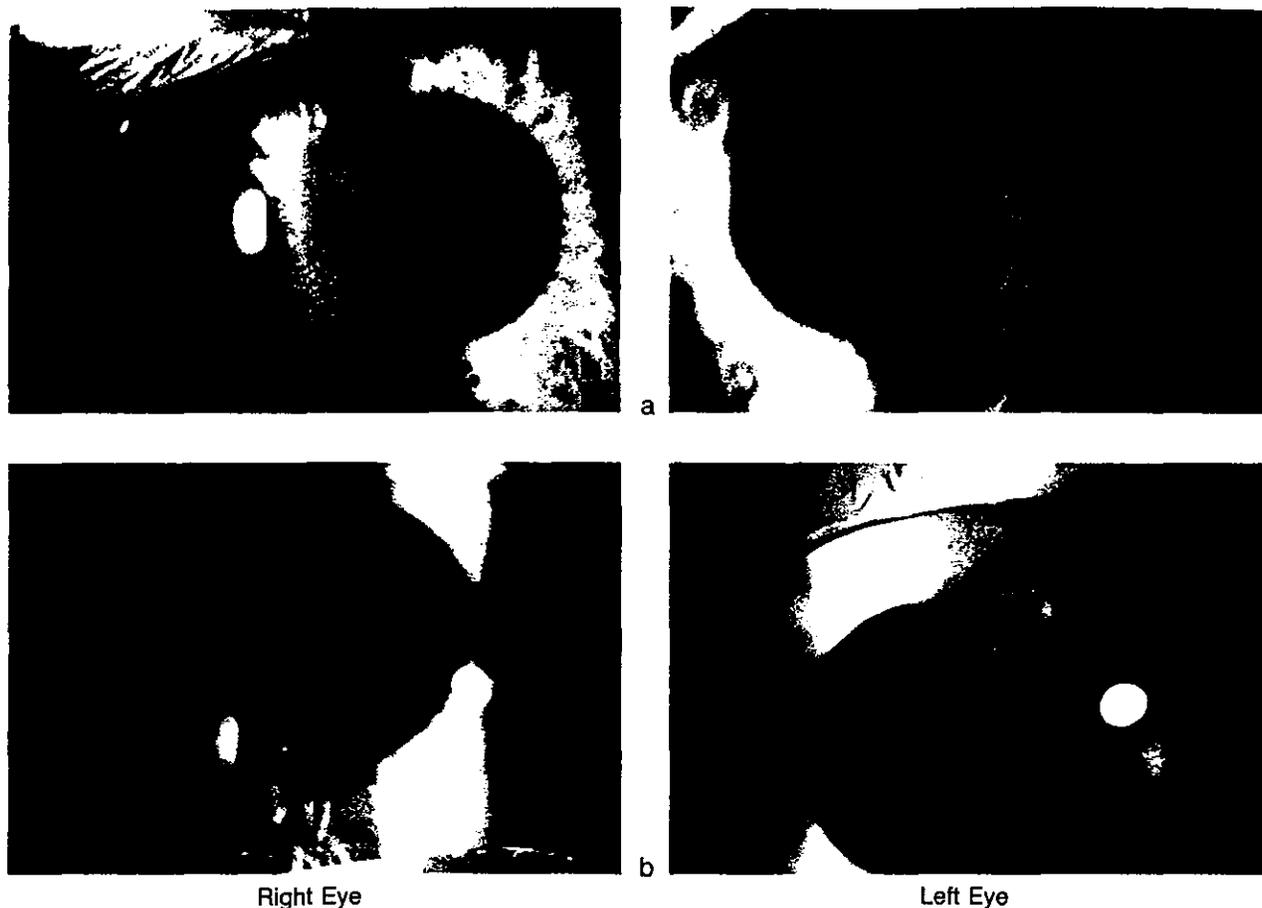


Figure 2. Slit-Lamp Appearance of the Corneas (Right Eye and Left Eye) of Patient 1.

The photographs in Part a were taken at 21 months of age, at the start of cysteamine-eyedrop treatment; note the bilateral, symmetrical crystal accumulation. As shown in Part b, after five months of treatment, the crystals had regressed markedly in the right eye but not in the left eye.

current treatment of the left eye. After approval was obtained from the National Eye Institute institutional review board, the drops being used in the right eye were also administered to the left eye. Three weeks later, the code for the eyedrops was broken to reveal that the right eye had been treated with cysteamine.

In Patient 2, corneal crystal accumulation progressed in both eyes between the ages of 13 months (Fig. 3a) and 19 months (Fig. 3b), one month before cysteamine treatment. After four months of treatment, the patient had a marked reduction in the number of corneal crystals in the right eye, whereas the number in the left eye was not markedly reduced (Fig. 3c). These findings were confirmed by four independent ophthalmologists who were unaware of which eye had been treated. Breaking the code revealed that the right eye had been treated with cysteamine.

No clinical toxicity was observed as a result of topical ophthalmic cysteamine. In particular, there was no conjunctival injection or soft-tissue irritation. Neither parents nor physicians could identify the cysteamine eyedrops by smell, apparently because the solution of free thiol was very dilute. Compliance was

determined to be excellent according to the history and review of the data forms on eyedrop administration maintained by the parents.

DISCUSSION

Cysteamine has been employed since 1978 as a cystine-depleting agent in cystinosis. Cysteamine passes through plasma and lysosomal membranes and, because it is a weak base, concentrates within acidic lysosomes. There, it reacts with cystine to form cysteine and a cysteine-cysteamine mixed disulfide, both of which traverse the cystinotic lysosomal membrane in a normal fashion.^{4,23} In fact, the mixed disulfide leaves by virtue of its steric resemblance to lysine, whose lysosomal membrane-transport system functions normally in cystinotic cells cultured *in vitro*.²⁴ *In vivo*, oral cysteamine readily depletes peripheral leukocytes of 90 percent of their endogenous cystine content¹⁶ and has proved efficacy in maintaining renal function and normalizing growth rates in children with cystinosis.¹⁷

Our experience in treating cystinotic children with oral cysteamine for up to eight years suggests that

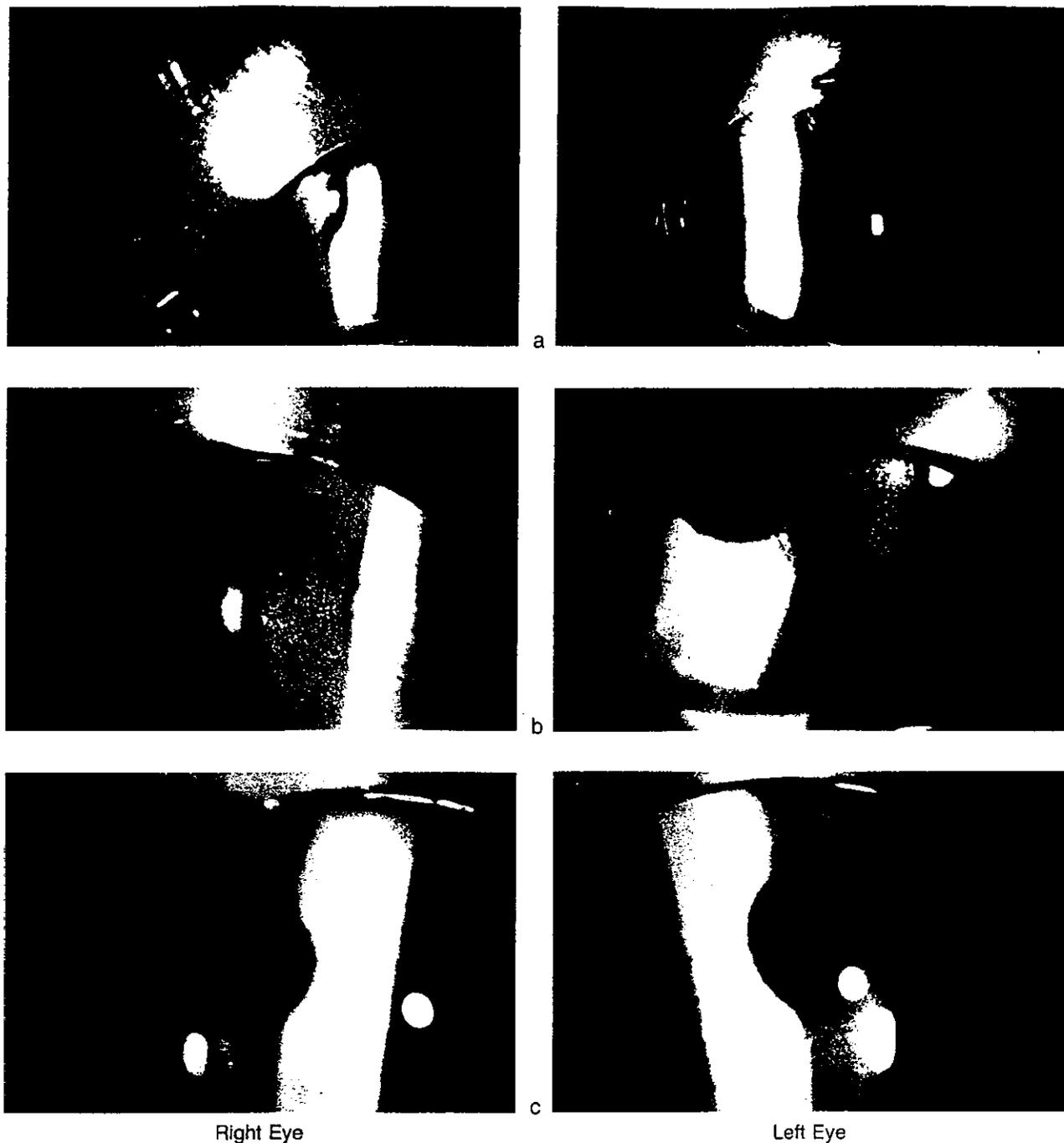


Figure 3. Slit-Lamp Appearance of the Corneas (Right Eye and Left Eye) of Patient 2 at 13 Months of Age (Part a, $\times 16$), 19 Months of Age, One Month before the Start of Treatment (Part b), and 24 Months of Age, after Four Months of Treatment (Part c).

Because of poor patient cooperation, corneal photographs taken at 20 months of age were inadequate. Note the progression of corneal crystals bilaterally between 13 and 19 months of age. After four months of cysteamine-eyedrop application, the right eye showed a marked diminution in crystal density.

systemic cysteamine does not prevent corneal crystal accumulation. However, corneal stromal cells from a patient with nephropathic cystinosis were substantially depleted of cystine by cysteamine *in vitro* (Fig. 1). In this respect they resembled cystinotic fibroblasts¹⁶ and renal cells²⁵ treated similarly. Moreover, *in vivo* studies revealed that both Patients 1 and 2 had an

absolute reduction in the number of corneal cystine crystals after topical cysteamine therapy for five and four months, respectively (Fig. 2 and 3). In fact, nearly all the crystals had been removed from the cysteamine-treated corneas (Fig. 2b and 3b).

The possibility remains that oral cysteamine reduced the number of corneal crystals in treated pa-

tients, but that the change was imperceptible against a background of hundreds of thousands of crystals per cornea. If so, the reduction in crystals attributable to oral cysteamine must have been minimal, since there was a negligible change in crystal density between 14 and 21 months of age in Patient 1. Furthermore, the number of corneal crystals actually increased between 13 and 19 months of age in Patient 2 (Fig. 3a and 3b).

One explanation for the difference in effectiveness between topical and oral cysteamine is derived from the fact that the cornea does not have a blood supply. Because of this, a cystine-depleting concentration of orally administered cysteamine could not reach corneal stromal cells; topical administration of cysteamine would obviate this problem. It should also be noted that the concentration of cysteamine in the eyedrops, 10 mM, is approximately 20 times the maximum level of cysteamine achieved in the blood of children receiving standard oral doses of the drug.²⁶ The very high local concentration of cysteamine at the cornea certainly contributed to its ability to dissolve the cystine crystals.

We deliberately selected young patients with cystinosis whose corneas were only moderately filled with crystals, so that the difference between the treated and untreated eyes would be apparent. The demonstrated efficacy of cysteamine eyedrops in these children does not mean that older cystinotic patients with packed corneas will realize the same benefit. In particular, we do not know what effect cysteamine eyedrops will have on the corneal clouding and recurrent corneal erosions that plague many patients with cystinosis after renal transplantation.^{9,15}

Two important findings follow from the results reported here. **First, the efficacy of cysteamine eyedrops in dissolving corneal crystals demonstrates that a cystinotic organ can be depleted of cystine (in crystalline or noncrystalline form) by cysteamine.** Previous *in vivo* investigations have been limited to demonstrating cystine depletion in circulating leukocytes.^{16,17} The present findings suggest that oral cysteamine, if adequately delivered to target tissues, could dissolve crystals in certain cystinotic organs, just as topically delivered cysteamine eyedrops removed cystine crystals from the cornea. Second, the lack of adverse reactions to topical cysteamine in animals and children and its cystine-depleting efficacy make cysteamine eyedrops the treatment of choice for corneal crystals in young children with cystinosis whose parents can comply with a fairly demanding regimen.

Despite these encouraging results, several questions require investigation. For example, is hourly administration required? Is 10 mM the optimal concentration of cysteamine to employ? Will older children benefit from this therapeutic regimen? Will our two patients avoid some of the later ocular complications of cystinosis, such as photophobia, corneal clouding, and recurrent corneal erosions, by virtue of cysteamine treatment of their corneas? The answers to these ques-

tions will require years of study, but we now have a strong impetus to pursue them.

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