Intravenous Cysteamine Therapy for Nephropathic Cystinosis

WILLIAM A. GAHL, JULIE INGELFINGER, PARVATHI MOHAN, ISA BERNARDINI, PAUL E. HYMAN, AND ALBERT TANGERMAN

Section on Human Biochemical Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892-1830 [W.A.G., I.B.], Department of Nephrology, Massachusetts General Hospital, Boston, Massachusetts 02115 [J.I.], Division of Gastroenterology and Nutrition, Children's National Medical Center, Washington, D.C. 20010 -2970 [P.M.], Department of Pediatric Gastroenterology, Children's Hospital of Orange County, Orange, California 92668 [P.E.H.], and Division of Gastrointestinal and Liver Diseases, University Hospital Nijmegen, Nijmegen, The Netherlands [A.T.]

ABSTRACT

A 4-y-old boy with nephropathic cystinosis and gastrointestinal dysmotility of unknown etiology was treated with i.v. cysteamine over a period of 10 mo. Thirty minutes after a dose of 10 mg/kg cysteamine free base, the leukocyte cystine value had fallen from 11.9 to 4.9 nmol of half-cystine/mg of protein. When cysteamine was given every 6 h, the leukocyte cystine concentration, measured 5–7 h after a dose, decreased with increasing cysteamine doses up to 17 mg/kg; at this dose the cystine value was 1.1 nmol of half-cystine/mg of protein, or 9% of the untreated value. Oral administration of approximately 16 mg/kg per dose every 6 h to this patient over the previous 3 y achieved similar leukocyte cystine depletion, to 1.2 nmol of half-cystine/mg of protein. The plasma cysteamine concentration 30 min after a dose of 10 mg/kg was 71 μM; 5–7 h after a dose of up to 20 mg/kg, the concentration was below 5 μM. Dimethylsulfide was elevated in the breath and urine of this boy after, but not before, the initiation of i.v. cysteamine therapy. Ten months after the start of therapy, the patient tolerated 250 mg (14 mg/kg) every 8 h. Adverse effects of this treatment included lethargy and increased nausea and vomiting when a schedule of therapy every 6 h was attempted. This investigation demonstrates that cysteamine given through a central venous catheter is effective in reducing leukocyte cystine levels. (*Pediatr Res* **38**: 579–584, 1995)

Abbreviation

X-S-SCH$_3$, the mixed disulfide of an unknown thiol and methyl sulfide

Nephropathic cystinosis is an autosomal recessive disease due to impaired transport of cystine across lysosomal membranes (1–4). The subsequent lysosomal storage of the poorly soluble cystine results in crystal formation and cellular damage in many tissues (5). The earliest involvement occurs in the renal tubules and causes Fanconi syndrome, with polyuria, dehydration, acidosis, rickets, and failure to thrive in infancy (6, 7). In untreated cystinosis, the inexorable progression of renal glomerular dysfunction leads to uremia and death by 9–10 y of age, unless dialysis or renal transplantation intervenes (6, 7). Cystinosis also results in growth retardation, hypothyroidism, photophobia, retinal damage, posterior synechiae and corneal ulcerations (8), pancreatic exocrine and endocrine insufficiency (9, 10), a distal vacuolar myopathy (11, 12), swallowing difficulties (13), and CNS involvement (14). These complications occur with variable frequencies and se-

verities, and at different times in life. However, virtually all posttransplant patients over age 30 suffer some major, debilitating complication of cystinosis (15).

The therapy of nephropathic cystinosis, besides replacement of renal losses, involves treatment with the cystine-depleting agent, cysteamine (16), or its more palatable bioequivalent, phosphocysteamine (17, 18). Cysteamine, given orally every 6 h at a dosage of 1.3–1.95 g/m^2/day (60–90 mg of free base/kg/day), has proven remarkably efficacious in preventing renal glomerular deterioration (19), permitting improvement of renal function in children under age 3 (20), and enhancing somatic growth in preadolescent cystinosis patients (19, 20). Long-term oral cysteamine therapy depletes cystinotic muscle of cystine (21), and topical cysteamine eyedrops dissolve corneal cystine crystals (22, 23). These demonstrations of parenchymal organ cystine depletion support a role for cysteamine in treating the nonrenal complications of cystinosis in posttransplant patients. Recently, cysteamine bitartrate capsules (Cystagon, Mylan Pharmaceuticals, Morgantown, WV) were approved by the

Food and Drug Administration for use in pretransplant cystinosis patients.

Among our patients with nephropathic cystinosis has been a 4-y-old boy who also suffers from gastrointestinal dysmotility refractory to pharmacologic intervention. Because this complication prevented the administration of cysteamine or phosphocysteamine through the gastrointestinal tract, and because a central venous catheter was already in place, we treated this patient with i.v. cysteamine at varying doses. We present data on the subsequent leukocyte cystine depletion as well as the concurrent plasma cysteamine concentrations achieved by this therapy.

METHODS

Intravenous cysteamine. Cysteamine for i.v. use was prepared by the National Institutes of Health Pharmaceutical Development Service under Investigational New Drug exemption no. 43770 sponsored by W.A.G. Informed consent was obtained from the patient's parents after a protocol was approved by the National Institute of Child Health and Human Development Institutional Review Board. Cysteamine (50 mg free base/mL) was diluted into a final volume of 50 mL and administered over 10–20 min into the patient's central venous catheter.

Assays. Leukocyte and tissue cystine concentrations were determined using the cystine binding protein assay (24); protein was measured using the bicinchoninic acid method (25). Plasma cysteamine was assayed by a method developed by Michael Adams and Paul Webster of Mylan Pharmaceuticals; Pharmaco LSR (Richmond, VA) validated and performed the assays. Briefly, plasma cysteamine was extracted with 8 M urea, derivatized with Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)], and analyzed by HPLC with UV detection. The minimal quantitation level was 0.100 μg/mL; the intraassay coefficient of variability was 5.7%. Breath dimethylsulfide was assayed by gas chromatography as previously described (26). Dimethylsulfide in urine was measured in the same way as previously reported for blood (27). In short, 2 mL of fresh urine was injected into a closed evacuated 15-mL glass vial. The urine was heated by hot tap water, and the headspace was sampled, concentrated onto Tenax, and investigated by gas chromatography. Methionine transamination metabolites were determined as previously described (28) as the sum of the mixed disulfides X-S-SCH$_3$ (serum, urine) and protein-S-SCH$_3$ (serum), and 4-methylthio-2-oxobutyrate (serum, urine).

CASE REPORT

The patient is a 4-y-old boy who had persistent fetal circulation at birth which required 5 d of extracorporeal membrane oxygenation for survival. In the first month of life, he fed poorly, required several formula changes, and failed to gain weight appropriately. His development otherwise proceeded normally, despite a prolonged episode of otitis media at age 3 mo and a urinary tract infection at age 8 mo. At the time of the urinary tract infection, he was found to have renal tubular Fanconi syndrome and rickets. A leukocyte cystine level of 10.5 nmol of half-cystine per mg of protein (normal, <0.2),

determined by Dr. Jerry Schneider, University of California-San Diego, secured the diagnosis of cystinosis. Corneal cystine crystals were not apparent at this time. Therapy with sodium citrate, phosphate supplements, and dihydrotachysterol was initiated. At 13 mo of age, oral phosphocysteamine therapy was begun and maintained until age 3½ y at a mean dosage of 1.30 g/m^2/day (64 mg of cysteamine free base/kg/day); this lowered the leukocyte cystine to a mean value of 1.2 nmol of half-cystine/mg of protein.

At 11 mo of age, a gastrostomy tube was placed due to the patient's frequent vomiting and poor feeding; subsequently, his food refusal worsened. A central venous catheter for total parenteral nutrition was placed in the superior vena cava at 14 mo of age and was followed by several episodes of sepsis. Gastrointestinal dysmotility was documented by manometry, but treatment with physostigmine and cisapride proved unsuccessful. Vomiting persisted, especially from midnight to noon, with some relief with ondansetron hydrochloride or metoclopramide. At age 3 y, an Arnold-Chiari malformation type I was diagnosed and treated with posterior fossa decompression, which resulted in temporary right-sided weakness and cyclic headaches. A karyotype performed at the 550 band level was normal.

On his sixth admission to the National Institutes of Health Clinical Center at age 4 y and 2 mo, the patient was able to take liquids, but would chew and spit out solids. Because he vomited several times a day and could not tolerate oral medications, phosphocysteamine had been discontinued approximately 6 mo before this admission. Nutrition was provided by total parenteral nutrition (2180 mL/day), although a jejunostomy tube was placed for some medications. On physical examination, the patient was a normal appearing, active blonde boy with a Hickman catheter in place. Height was 97.8 cm (5–10 centile), weight 15.1 kg (10–25 centile), and head circumference 51.5 cm (50–75 centile). The remainder of the examination was unremarkable. Serum sodium was 138 mEq/L, potassium 3.4 mEq/L, chloride 104 mEq/L, and carbon dioxide 28 mEq/L. Serum creatinine was 1.1 mg/dL, blood urea nitrogen 7 mg/dL, alkaline phosphatase 230 U/L, alanine aminotransferase 14 U/L, aspartate aminotransferase 41 U/L, albumin 4.4 g/dL, calcium 2.59 mmol/L, magnesium 0.83 mmol/L, and thyroxine 11.7 μg/dL. The Hb was 7.4 g/dL, hematocrit 20.1%, and platelet count 95,000/mm^3. The white blood cell count was 2500/mm^3, with 43% polymorphonuclear cells, 44% lymphocytes, 6% mononuclear cells, 6% eosinophils, and 1% basophils. The reticulocyte count was 2%. The erythropoietin level was 5 mU/mL, inappropriately low for the patient's degree of anemia. A bone marrow biopsy and aspirate revealed a myeloid/erythroid ratio of 1.3/1 with diffuse lymphocytosis and focal eosinophilia, mild megaloblastic granulopoiesis, and crystal-laden macrophages. Two creatinine clearance measurements were low, *i.e.* 27 and 48 mL/min/1.73 m^2.

At 4%₁₂ y of age, manometry was performed by one of us (P.E.H.). Fasting and postprandial antroduodenal manometry was dominated by normal amplitude propagating and non-propagating clustered contractions, a pattern of repetitive discreet abnormalities typical of a neuropathic intestinal motility disorder. Colonic manometry was normal.

RESULTS

Gastrointestinal biopsies. A rectal biopsy performed at 3 y and 4 mo revealed typical cystine crystals within fibroblasts and histiocytes of the lamina propria (Fig. 1). In addition, noncrystalline deposits and membranous whorls were evident. The biopsy contained 3.0 nmol of half-cystine/mg of protein (normal large intestine (Brain and Tissue Bank, University of Miami), 0.064 ± 0.036 SEM nmol of half-cystine/mg of protein, $n = 3$). Subsequently, at 4 y of age, biopsies of the esophageal, gastric, duodenal, and rectal mucosa were also performed. Crystals were apparent on light and electron microscopy within the macrophages of the lamina propria in the duodenum (Fig. 2). No other specific pathology was observed. Ganglion cells were present in the rectal biopsy. The rectal mucosa contained 0.37 nmol of half-cystine/mg of protein and the submucosa contained 0.23 nmol of half-cystine/mg of protein.

Intravenous cysteamine. Intravenous cysteamine was initiated at age 4 y and 2 mo because of inability to tolerate enteral cysteamine. The patient received increasing doses of cysteamine through his central line, each given in 50 mL of normal saline over approximately 20 min. At 5 or 10 mg/kg per dose (75 or 150 mg of free base), i.v. cysteamine lowered leukocyte cystine values (baseline, 11.9 nmol of half-cystine/mg of protein) approximately 50% within 30 min and maintained this depletion for up to 6 h (Fig. 3). In fact, leukocyte cystine concentrations 5–7 h after a dose of cysteamine fell progressively with increasing doses of the drug (up to 17 mg/kg), leveling off at 1.1 nmol of half-cystine/mg of protein, or 9% of baseline (Fig. 4).
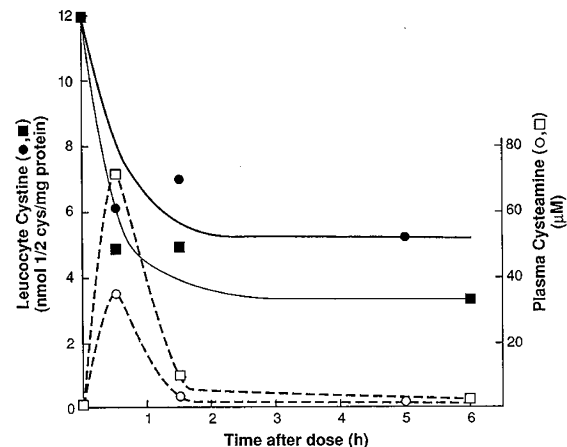
Peak plasma cysteamine levels after doses of 5 or 10 mg/kg were observed 30 min after a cysteamine injection, and reached 35 and 71 μM, respectively (Fig. 3). The half-times for loss of cysteamine from the plasma, based upon the 30- and 90-min values, were 18 min for the 5 mg/kg dose and 20 min for the 10 mg/kg dose. Five to seven hours after a cysteamine dose of 5 mg/kg, plasma cysteamine was undetectable (<1.3 μM).
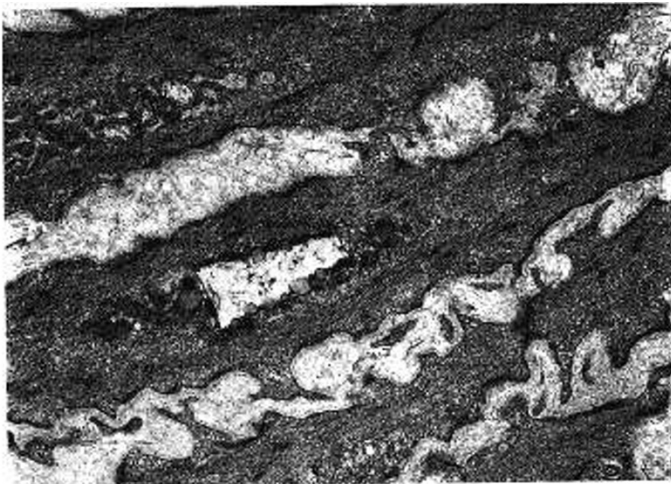
Figure 1. Electron micrograph of rectal biopsy at 3⁴/₁₂ y of age. (Courtesy of Dr. James Southern, Department of Pathology, Massachusetts General Hospital.) Crystal surrounded by vacuoles storing amorphous noncrystalline material (×9100).


Figure 2. Electron micrograph of duodenal biopsy performed at 4 y of age. (Courtesy of Dr. Dena M. Selby, Attending Pathologist, Children's National Medical Center.) A macrophage in the lamina propria shows a single intracytoplasmic cystine crystal (×5200).

Figure 3. Leukocyte cystine levels and plasma cysteamine concentrations after i.v. doses of cysteamine. A single dose of 5 mg/kg (75 mg of cysteamine-free base, ○, ●) or 10 mg/kg (150 mg of cysteamine-free base, □, ■) was injected into the patient's central line in a volume of 50 mL of normal saline over 20 min, and blood was sampled after various times. Single leukocyte cystine determinations are represented by *filled symbols*; plasma cysteamine values are *open symbols*. The lower limit of detection for plasma cysteamine is 1.3 μM.

However, doses of 10–20 mg/kg yielded increasing plasma cysteamine concentrations 5–7 h later, ranging from 1.9 μM for 10 mg/kg to 4.1 μM for 20 mg/kg (Fig. 4). (Note the 20-fold increased scale in Fig. 4 compared with that in Fig. 3.)

Dimethylsulfide in both breath (<0.1 nmol/L) and urine (<5 nmol/L) were undetectable in the patient before cysteamine therapy. However, after the patient received 60–68 mg/kg/day of i.v. cysteamine for 9 d, breath dimethylsulfide was 8.35 nmol/L and urine dimethylsulfide, 500 nmol/L. Dimethyldisulfide, 0.5 nmol/L (normal, zero), was also detected in the breath after i.v. cysteamine therapy. However, the methionine transamination metabolites (mixed disulfides and X-S-SCH₃, protein-S-SCH₃, and 4-methylthio-2-oxobutyrate) remained normal in both urine (before cysteamine, 0.51 mmol/mol creatinine; after, 2.37 mmol/mol creatinine; normal, 0.7–3.2 mmol/mol creatinine) and serum (before cysteamine, 0.31

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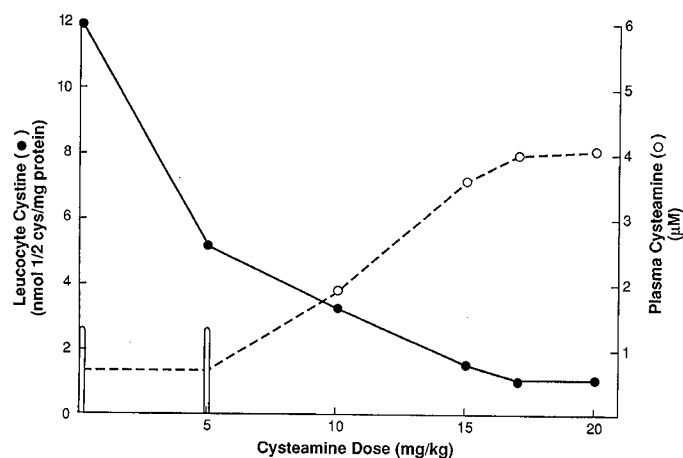
Figure 4. Leukocyte cystine (●) and plasma cysteamine (○) concentrations after various doses of i.v. cysteamine. In all cases, blood was drawn 5–7 h after the dose of cysteamine, *i.e.* at the nadir for plasma cysteamine and just before the next dose. After doses of 0 or 5 mg/kg, the plasma cysteamine concentration was below the assay's detection limit (1.3 μM), as signified by the *open bars*. Symbols represent single determinations.

μmol/L; after, 0.47 μmol/L; normal, 0.20–0.50 μmol/L). The patient emitted a sulfur-like breath odor similar to the odor he had previously displayed on oral cysteamine.

Long-term therapy. Intravenous cysteamine therapy was continued at home at doses of 12–14 mg/kg every 6 h. After 3 mo, the mean leukocyte cystine value was 1.4 nmol of half-cystine/mg of protein and the mean plasma cysteamine was 2.5 μM 5–7 h after a dose. After 8 mo, the mean leukocyte cystine value was 2.0 nmol of half-cystine/mg of protein. However, in general, therapy could be given only every 8 h owing to nausea, vomiting, and lethargy which became nearly constant when i.v. cysteamine was given at 6-h intervals. Ten months after the initiation of i.v. cysteamine, the patient was able to tolerate 250 mg (14 mg/kg) every 8 h with vomiting approximately three times per day. In addition, the patient was able to take 50% of his calories by jejunostomy tube while on scheduled ondansetron hydrochloride, 4 mg t.i.d.

The mean creatinine clearance in the 2 y before i.v. cysteamine therapy was 37.1 ± 9.5 mL/min/1.73 m^2 ($n = 8$); the mean creatinine clearance in the 8 mo after i.v. cysteamine therapy was 34.5 ± 8.7 mL/min/1.73 m^2 ($n = 4$).

DISCUSSION

Various types of gastrointestinal involvement have been recognized in nephropathic cystinosis, including ulcerative colitis in a single patient (29) and cystine accumulation in several affected children (30, 31). In these individuals, cystine crystals were demonstrated within histiocytes of the rectal lamina propria, corresponding to the location of crystals in our patient's rectal mucosa. Intestinal cystine concentrations in cystinosis patients have not been previously reported, but our patient's values were clearly elevated at 3 nmol of half-cystine per mg of protein. On the other hand, this boy's rectal mucosal cystine concentration was considerably less than that of another 6-y-old cystinosis patient (182 nmol of half-cystine/mg of protein) (Gahl W, unpublished data), who had normal

gastrointestinal motility. Consequently, we cannot attribute our patient's gastrointestinal dysmotility to cystine accumulation or to any aspect of his cystinosis. Although many children with cystinosis have nausea and vomiting, none has had the severe, intractable problems experienced by our patient. Additionally, Arnold-Chiari malformation and persistent fetal circulation complicated his history and suggest other etiologies for the unusual course of his disease. We also cannot blame cysteamine therapy itself; although cysteamine is a duodenal ulcerogen in rats (32), we know of no reports of gastrointestinal dysmotility resulting from the therapy. In fact, the patient's dysmotility preceded the initiation of cysteamine therapy.

Whatever the cause, this boy's gastrointestinal dysfunction in the face of cystinosis called for administration of parenteral cysteamine. To our knowledge, there has been only one other report involving the use of i.v. cysteamine (16). A single i.v. dose of 10 mg/kg was administered to a 7-y-old girl over a 5-min period; 1 h later, the leukocyte cystine concentration was 30% of the initial value. Plasma cysteamine determinations were not available.

Our patient underwent incremental dosing of i.v. cysteamine to a maximum of 20 mg/kg per dose, with leukocyte cystine levels decreasing as the dose was increased (Fig. 4). Moreover, the response of the leukocyte cystine concentration to i.v. cysteamine resembled that typical for oral cysteamine. For example, we noted a 62% leukocyte cystine depletion 90 min after an i.v. cysteamine dose of 10 mg/kg; Smolin *et al.* (18) found 62% depletion 1 h after a dose of 18 mg/kg. With our patient serving as his own control, leukocyte cystine concentrations at their nadir (approximately 6 h after a dose) were virtually identical whether the dose was received i.v. or orally. Specifically, when 68 mg/kg/day was given i.v., the leukocyte cystine was 1.1 nmol of half-cystine/mg of protein and when 64 mg/kg/day was given orally, the value was 1.2 nmol of half-cystine/mg of protein. This suggests virtually complete gastrointestinal absorption of cysteamine.

This observation finds further support in comparison of peak plasma cysteamine concentrations after i.v. and oral doses of cysteamine. Smolin *et al.* (18) administered 0.23 mmol/kg (18 mg/kg) of cysteamine orally to six children with cystinosis age 2–10 y and reported peak plasma cysteamine concentrations of 34.1–62.7 μM (mean ± SD, 48.6 ± 10.7 μM) 30–60 min later. (Oral phosphocysteamine gave virtually the same results as cysteamine itself.) Jonas and Schneider (33) administered 0.14–0.23 mmol/kg (11–18 mg/kg) per dose to five cystinosis patients age 1–9 y and reported plasma cysteamine concentrations of 14–54 μM 1 h later. Our patient's plasma cysteamine concentration 30 min after an i.v. dose of 10 mg/kg was 71 μM. In Jonas and Schneider's study, plasma cysteamine concentrations ranged from undetectable (<5 μM) to 18 μM 6 h after a dose; in our patient, the plasma cysteamine averaged 3.6 μM 5–7 h after a dose of 15 mg/kg.

The relatively short half-life of cysteamine in plasma, approximately 20 min, reflects its conversion to excretable sulfur-containing compounds. Jonas and Schneider (33) have reported only 0.3–1.7% of an oral dosage of 6.6–15.8 mmol/day excreted intact in the urine, indicating that other metabolites must be involved. One such product is dimethylsulfide, a volatile

compound that was also highly elevated in a breath sample taken 9 d after the initiation of cysteamine therapy. Dimethylsulfide did not make a significant contribution to the patient's urinary sulfur excretion, because it amounted to less than 1 μmol/day. However, its expiration via this patient's breath (8.35 nmol/L) would approximate 0.3 mmol daily. This amounts of 3.1% of the total cysteamine dose (approximately 9.7 mmol/day). The contribution of the proposed pathway might be much higher than 3.1%, however, because a large portion may have escaped detection via other metabolites of methanethiol, e.g. sulfate (34).

More important, the presence of dimethylsulfide in our patient's breath and urine may indicate a new pathway in cysteamine catabolism. To our knowledge, dimethylsulfide has not previously been identified as a metabolite of cysteamine. We propose that cysteamine is first methylated to the thioether (35), followed by a cytochrome P-450-mediated S-dealkylation of the thioether to methanethiol (36) and a second methylation to dimethylsulfide (Fig. 5). That methanethiol is an intermediate is supported by the presence of small amounts of dimethyldisulfide, the oxidation product of methanethiol, in the patient's breath. The breakdown of cysteamine to dimethylsulfide and dimethyldisulfide must be of mammalian origin and could not have occurred due to a chemical breakdown of cysteamine before its infusion because neither dimethylsulfide, dimethyldisulfide, nor methanethiol was present in the cysteamine solution before infusion. The proposed pathway of dimethylsulfide formation would not have been attributed to human metabolism if the cysteamine had been administered orally, because gut organisms would have been blamed for the products formed.

The main adverse effect of i.v. cysteamine observed in this study was an increased frequency of vomiting and the occurrence of lethargy at high doses, typical of oral cysteamine. The fact that vomiting was exacerbated by i.v. cysteamine indicates that its emetic effects are not entirely due to its putrid smell and repulsive taste, nor due to direct gastrointestinal upset. Rather, cysteamine appears to be acting centrally, eliciting an emetic response at high plasma concentrations. This is consistent with the occasional somnolence and lethargy observed with high doses of oral cysteamine (37). An additional nuisance associated with i.v. or oral cysteamine is an offensive breath odor, here attributable to dimethylsulfide. A similar finding has been reported in a 31-y-old man with methionine adenosyltransferase deficiency (38).

The pharmacokinetic findings in our patient may not be directly applicable to other children or adults with cystinosis.
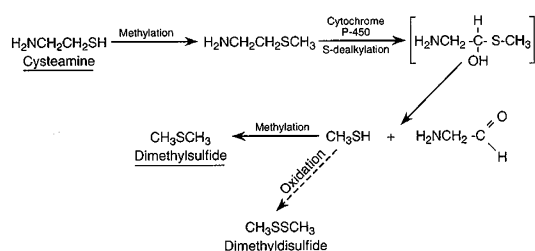
In addition, i.v. cysteamine therapy should be reserved for the rare or, perhaps, unique case in which gastrointestinal dysmotility prevents the alimentary tract from providing access for nutrition and medication. Ordinarily, a child with cystinosis who vomits excessively and does not tolerate oral cysteamine can still be nourished and treated through a gastrostomy or jejunostomy tube.

Cysteamine is now approved for use only in pretransplant cystinosis patients, although its use in posttransplant patients has been proposed to prevent late complications of the disease (8-15, 21). Nevertheless, the theoretical applications of cysteamine are quite extensive. Whenever an arginine to cysteine substitution occurs in a circulating protein, cysteamine has the potential to convert the cysteine back to an arginine or lysine analog by forming the mixed disulfide cysteine-cysteamine. Examples include apolipoprotein E_2 (39) and anti-thrombin III_{Toyama} (40). Another possible therapeutic use of cysteamine is in human immunodeficiency virus infections, because cystamine, the disulfide of cysteamine, has recently been shown to inhibit human immunodeficiency virus replication in a cell culture system (41).

Figure 5. Proposed scheme of dimethylsulfide synthesis from cysteamine.

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