0031-3998/85/1911-1170$02.00/0
PEDIATRIC RESEARCH

Vol. 19, No. 11, 1985
Printed in U.S.A.

Lysosomal Cystine Storage in Cystinosis and Mucolipidosis Type II[1]

ALICE A. GREENE, ADAM J. JONAS,[2] ERIK HARMS,[3] MARGARET L. SMITH, OCEAN L. PELLETT, EDWARD A. BUMP,[4] ARNOLD L. MILLER, AND JERRY A. SCHNEIDER

Department of Pediatrics [A.A.G., M.L.S., O.L.P., J.A.S.] and Department of Neurosciences [A.L.M.], University of California, San Diego, School of Medicine, La Jolla, California 92093

ABSTRACT. Cultured fibroblasts from mucolipidosis II (ML-II) patients demonstrated an elevated cystine content which increased with time in culture compared to fibroblasts from cystinotic patients or normal controls under the same conditions. In both cystinotic and ML-II cells the increased levels of cystine could be derived either from endogenous proteolysis or from *in vitro* supplementation of the cultured cells with cysteine-glutathione mixed disulfide. Cystine was depleted from both cell types by cysteamine. When cysteamine was replaced with complete medium, the cystine reaccumulated in both cystinotic and ML-II cells within 24 h, although a lag of 4 h was seen with ML-II cells. The intracellular location of the increased cystine in cultured fibroblasts was examined utilizing free-flow electrophoresis and found to be in the purified population of secondary lysosomes of both cystinotic and ML-II cells. White blood cell and hepatic cystine, which was greatly increased in cystinotic patients, was not elevated in ML-II patients. Compared to normal control fibroblasts the efflux of cystine from isolated granular fractions was virtually absent in cystinotic fibroblasts and considerably reduced in ML-II fibroblasts. The examination of such similarities and differences in cystine accumulation and transport in tissues from cystinotic and ML-II patients has provided some insight into the defects in these diseases. (*Pediatr Res* 19: 1170–1174, 1985)

Abbreviations

ML-II, mucolipidosis type II
PBS, phosphate buffered saline

The autosomal recessive disorder, cystinosis, is characterized by the lysosomal storage of the disulfide amino acid cystine in many different tissues including kidney, liver, leucocytes, and cultured skin fibroblasts (1–3). Cystine efflux from the lysosomes has been found to be defective in cells from cystinotic patients (4–9). The inability to transport cystine appears to be the result of a defect in a cystine transport protein (10). Cystine also accumulates in cultured fibroblasts from individuals with ML-II (1, 11). When first reported this raised the possibility that the defect causing cystine accumulation was the same in both diseases. It is now known that the defect in ML-II is a diminished activity of N-acetylglucosamine 1-phosphotransferase which catalyzes the addition of the mannose-6-phosphate marker to enzymes targeted for the lysosomes. Such a marker might also be required for delivery of the cystine transport protein to the lysosomal membrane. A recent report by Steinherz *et al.* (1) concludes that although cystine accumulates in ML-II cultured fibroblasts, the ability to clear cystine is not impaired. In contrast to this report (1) we find that lysosomal cystine efflux is defective in cultured ML-II fibroblasts although not to the degree found in cystinotic cells.

METHODS

All chemicals and enzyme substrates, unless otherwise noted, were obtained from Sigma Chemical Co. L-^{35}S-cystine, 119 mCi/mmol, was obtained from Amersham Co. Omnifluor was obtained from New England Nuclear. Coon's modified F12 medium (12) was purchased from K. C. Biological and Irvine Scientific. Fetal bovine serum was obtained from Flow Laboratories and Armour Pharmaceutical Co. Trypsin (0.05%) in buffered saline with 0.02% EDTA was purchased from Irvine Scientific. ^{35}S-cystine dimethyl ester was prepared as previously described (8). Inorganic reagents for the leucocyte preparation were obtained from Mallinckrodt and the dextran (molecular weight 200,000–275,000) from Polyscience. Cysteine glutathione disulfide was prepared by the reaction of cystine thiosulfonate with glutathione and purified by ion exchange chromatography (13). Cysteamine, chloroquine, and the mixed disulfide of cysteine and glutathione were added to culture medium as freshly prepared, neutralized solutions. Culture medium was sterilized by filtration through a 0.22 μ Millipore filter.

Cell lines were established from skin biopsies of four patients homozygous for ML-II, three patients homozygous for infantile, nephropathic cystinosis, and three normal, age-matched controls. Cells were grown in either 100-mm plastic dishes or glass roller bottles using Coon's modified F12 medium supplemented with 2.0 mM glutamine and 10% fetal bovine serum (12). The cells were placed in a 37° C incubator with an atmosphere of 10% CO_2 and 90% air.

Except for transport studies, cells were harvested using trypsin and were prepared for cystine analysis as previously described (14). Cystine was measured using a specific binding protein assay (14), and cellular protein was dissolved in 0.1 N NaOH and determined spectrophotometrically (15). Cells to be depleted of cystine were first washed with PBS (16) and then placed in cystine-free, serum-free medium containing cysteamine. After

Received April 24, 1985; accepted June 25, 1985.
Jerry A. Schneider, M.D., Department of Pediatrics, M-009-F, University of California, San Diego School of Medicine, La Jolla, CA 92093.
Supported by The March of Dimes Grant 1-880 and The Cystinosis Foundation, Deutsche Forschungsgemeinschaft Grants Ha 756/2-756/6, National Research Service Award 5F32AM06241, and National Institutes of Health grants AM 18434, GM 17702, AM 01074, NS 12138.

[1] Parts of this study were presented to the Western Society for Pediatric Research in Carmel, CA, 1980 and to The Society for Pediatric Research in San Francisco, CA 1981.

[2] Present address: Department of Pediatrics, Medical School, University of Texas, Houston, 6431 Fannin Street, Houston, TX 77030.

[3] Present address: Universitats-Kinderklinik, 6900 Heidelberg, West Germany.

[4] Present address: Department of Radiology, Stanford University Medical Center, Stanford, CA 94305.

treatment with cysteamine, the culture medium was removed and the cells washed with PBS prior to harvest.

Highly purified lysosomes were prepared from approximately 4×10^7 cells grown for 24 h in medium containing 1 mCi/100 ml of ^{35}S-cystine. The cells were harvested and fractionated as previously described (17). Lysosomal purification was performed using a Bender and Hobein, VaP5 free-flow electrophoretic apparatus (17). Fractions obtained from free flow electrophoresis were assayed for protein content spectrophotometrically (18, 19). Lysosomal fractions were identified by β-glycerophosphatase (E.C. 3.1.3.2) activity (20) which is not decreased in ML-II cell lysosomes (21) while succinate-3-(4-iodophenyl)2-(4 nitrophenyl)-5-phenyl-2H-tetrazolium chloride(INT) reductase (E.C. 1.3.99.1) activity (22) was used to identify mitochondrial fractions. Lysosomal fractions were pooled and collected by centrifugation at 20,000 × *g* for 10 min at 4° C. The lysosomal pellet was sonicated in 100 μl of 10 mM potassium phosphate, pH 7.2 with 5 mM N-ethylmaleimide and mixed with 50 μl of 12% sulfosalicylic acid. The acid-soluble labeled compounds were separated by high voltage paper electrophoresis (23). Compounds of interest were cut out of the chromatogram and quantitated by scintillation counting using Omnifluor.

Leucocytes were prepared by mixing fresh, heparinized blood with an equal volume of dextran-acid citrate dextrose solution. The dextran-ACD solution consisted of 3 g dextran, 2.1 g dextrose, 0.33 g sodium citrate, and 0.11 g anhydrous citric acid in 100 ml of normal saline. After settling for 30 min at room temperature, the supernatant was removed and centrifuged at 450 × *g* for 10 min. The leucocyte pellet was saved and suspended in 0.22% NaCl for 90 s to lyse hypotonically any remaining red cells. The salt concentration was then adjusted to 0.9% by the addition of 3.6% NaCl. The cells were collected by centrifugation at 450 × *g* for 3 min. The hypotonic lysis was repeated if necessary. The leucocyte pellet was washed twice more with 3 ml of normal saline. The pellet was sonicated in 0.3 ml H_2O and mixed with 0.1 ml of 12% sulfosalicylic acid. Cystine determinations were performed on the acid-soluble fraction (14) while protein was measured on the acid-insoluble fraction (15).

Human liver was obtained from autopsy specimens and stored frozen at −20° C. A portion of each liver was weighed and then dispersed by sonication in 0.9 ml of 10 mM potassium phosphate buffer, pH 7.2 with 10 mM N-ethylmaleimide. The resulting suspension was mixed with 0.3 ml of 12% sulfosalicylic acid and centrifuged at 20,000 × *g* for 20 min at 4° C. The supernatant was analyzed for cystine content while the pellet was assayed for protein content as above.

Cystine transport studies were performed on partially purified lysosomes. Normal cells, grown to confluency in roller bottles, were washed and harvested by scraping in PBS at 30° C. Cells were collected by centrifugation and resuspended in serum-free Coon's modified F12 without cystine with 0.5 or 1.0 mM cystine dimethyl ester. The cells were incubated at 37° C, for 15 min while shaking. The cells were pelleted, washed twice with cold PBS, and used for preparation of granular fractions as previously described (9). Cystinotic cells were prepared as above except no cystine dimethyl ester was present during the incubation at 37° C. Cellular cystine level of cystinotic cells was lowered prior to harvesting by incubation for 14 h in cystine free medium with 10% dialysed fetal bovine serum. ML-II cells were grown 2–8 wk past confluency, then prepared as the normals, but without cysteine dimethylester. Cystine efflux studies were performed as described previously (9). Lysosomal cystine was expressed as nmol of cystine per mg of protein. Latency was assessed by measurement of β-hexosaminidase (E.C. 3.2.1.20) activity of the pelleted and resuspended granules at the beginning and end of the efflux period. Cystine values were corrected for any lysosomal breakage by normalizing to constant β-hexosaminidase. Although diminished in ML-II cells, β-hexosaminidase is still useful as an indicator of lysosomal integrity.

RESULTS

The cystine content of ML-II cultured fibroblasts increased with time in culture, between 1 and 3 wk after passage, approximately 7-fold to the range found in cystinotic fibroblasts (Table 1). The cystine content of cystinotic and normal control fibroblasts did not increase significantly during the same period.

Fibroblast lysosomes from cells grown in ^{35}S-cystine were purified 12- to 16-fold by free flow electrophoresis. The pattern of distribution of radiolabel, protein, and various enzyme activities in normal, ML-II, and cystinotic cell organelles is seen in Figure 1. The peak corresponding to lysosomes in the ML-II electrophoretic pattern runs closer to the anode compared with that of the normal and cystinotic reflecting a more negative surface charge analogous to previous results (24). Lysosomal cystine was 50-fold higher in ML-II cells than in normal cells (Table 2).

Exposure to 5 mM cysteamine for 1 h induced an 80% loss of cystine in ML-II cells and a 90% loss in cystinotic cells. Microscopic examination of cells exposed to 5 mM cysteamine revealed normal morphology. Following cysteamine treatment, cells were washed and placed in fresh, complete culture medium with 10% fetal bovine serum. Over the next 4 h, cystinotic cells rapidly reaccumulated cystine while ML-II cells reaccumulated little or no cystine (Fig. 2). Over the next 4–20 h, however, the rates of cystine reaccumulation were similar in both cell types. The reaccumulation by both cystinotic and ML-II fibroblasts was enhanced in the presence of the cysteine-glutathione mixed disulfide and inhibited by 50 μM chloroquine (Table 3).

Leucocyte cystine levels of a child with ML-II were within the normal range of <0.1 nmol/mg protein. In contrast, leucocyte cystine in cystinotic individuals ranges from 3–10 nmol/mg protein.

Liver cystine levels of three ML-II patients were also in the range of a control done at the same time (0.1–0.2 nmol/mg protein), whereas the hepatic cystine content of a cystinotic sample was 10.9 nmol/mg protein.

ML-II cells were grown under conditions which enhanced the accumulation of cystine. The lysosomal cystine efflux from ML-II cells was then compared with that from cystinotic cells of comparable cystine content as well as with that from cystine loaded normal cells (Fig. 3). As a control, cystinotic and some of the ML-II cell preparations were carried through the loading procedure in the absence of the cystine dimethyl ester.

The rate of cystine loss from normal control fibroblast lysosomes was greater than the loss from ML-II fibroblast lysosomes and much greater than that from cystinotic fibroblast lysosomes (Fig. 3). The efflux of cystine from normal and ML-II fibroblast lysosomes was approximately proportional to the cystine level. ML-II cell strains differed in amount of cystine accumulated and efflux rates. The cell strain MD could not be distinguished from cystinotic cells with respect to efflux, yet this strain never accumulated more than 2 nmol cystine/mg even after 52 days past confluence.

DISCUSSION

Although this study demonstrates that lysosomal cystine egress is defective in ML-II fibroblasts, it does not pinpoint any one

Table 1. *Cystine content of cultured ML-II fibroblasts*

	Cellular cystine (nmol cystine/mg protein)	
	Wk 1	Wk 3
Normal	0.2 ± 0.1*	0.2 ± 0.1
Cystinosis	11 ± 1	15 ± 4
ML-II	0.9 ± 0.4	6.3 ± 3.4

* Mean ± SD.

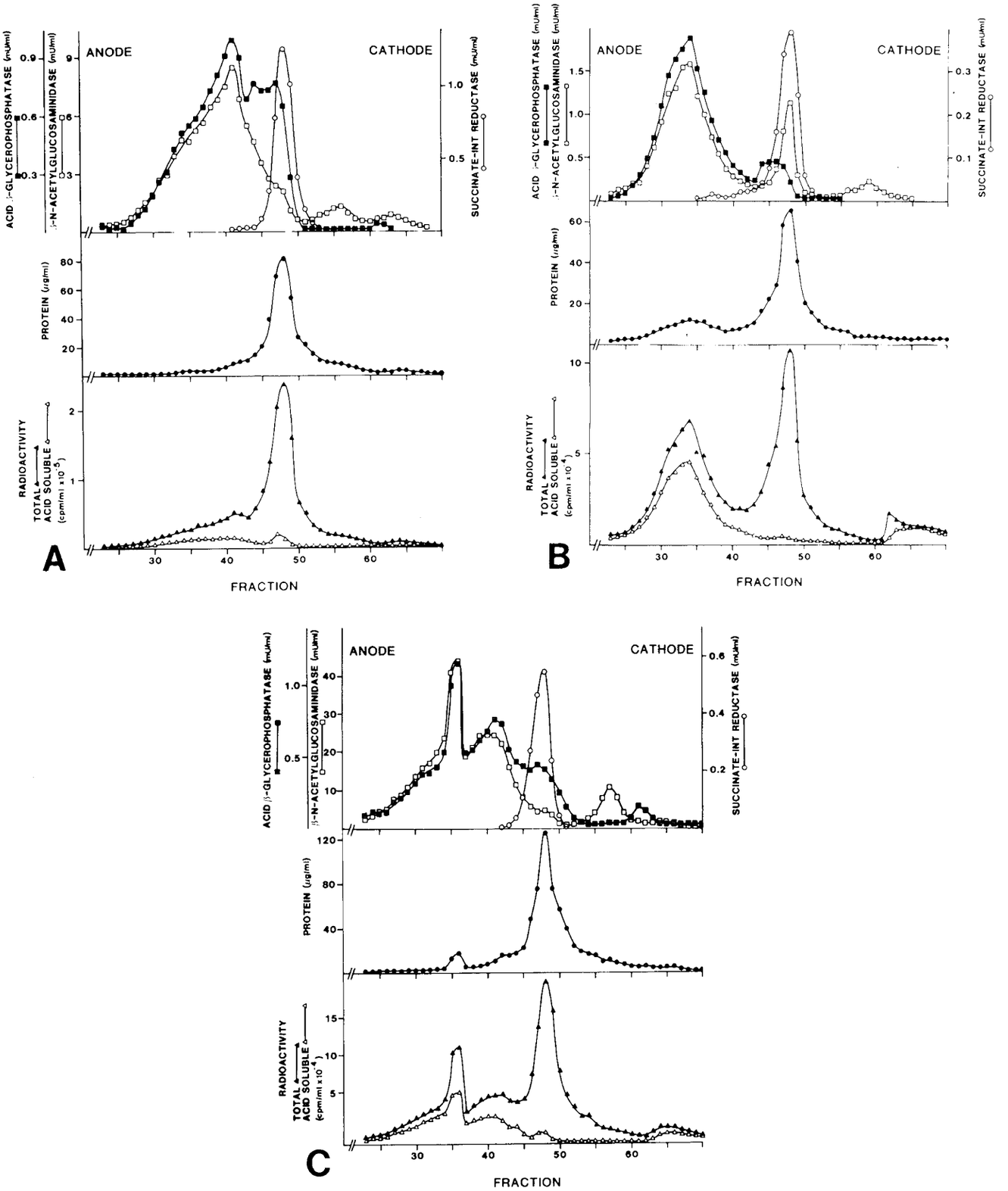

Fig. 1. Free flow electrophoretic separation of ML-II fibroblast intracellular organelles. Cells had been grown for 24 h in ^{35}S-cystine before separation. Mitochondrial and lysosomal fractions enzymatic activities and counts as ^{35}S-cystine were determined as described in methods. *A*, normal; *B*, ML-II; *C*, cystinosis. (■) acid β-glycerophosphatase mU/ml, (□) β-N-acetylglucosaminidase mU/ml, (○) succinate-INT-reductase mU/ml, (●) protein μg/ml, (▲) total radioactivity, (△) acid soluble radioactivity cpm/ml × 10^{-4}.

specific cause for this defect. The first possibility to consider is that the cystine transport protein, like many lysosomal enzymes, may not be normally targeted to lysosomes in ML-II fibroblasts. This study has demonstrated many similarities between ML-II fibroblasts and cystinotic fibroblasts which are defective in cystine transport activity (1–11). The cystine in ML-II cells is lysosomal and, like cystinotic cells (4, 26), the cystine accumulation is increased by cysteine glutathione disulfide and inhibited

Table 2. *Free flow electrophoretic purification of ^{35}S-labeled lysosomes**

	β-Glycerophosphatase (U/mg protein)	Lysosomal cystine (cpm × 10^{-3}/mg protein)
Normal	110	31
Cystinosis	60	2400
ML-II	153	1500

* Lysosomes from fibroblasts grown in medium containing ^{35}S-cystine were purified by free flow electrophoresis. Purified lysosomes were assayed for β-glycerophosphatase and protein as in "Methods." Acid soluble lysosomal contents were assayed for cystine by high voltage paper electropohoresis.

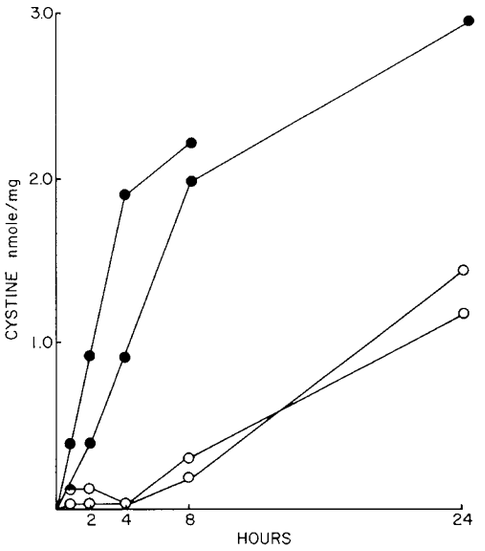

Fig. 2. Cystine reaccumulation by ML-II (○) and cystinotic (●) cells was examined following treatment for 1 h with 5 mM cysteamine in cystine-free medium without serum. Cells were washed with 5 ml of phosphate-buffered saline and then placed in complete medium with 10% fetal bovine serum. The cystine content of duplicate plates of cells was determined during a 24-h period. Each value represents the observed cystine content minus the cystine content immediately following treatment with cysteamine. Cystine is expressed as nmol/mg of cellular protein. Zero-time values for cystinotic cells were 0.1 and 0.3 nmol cystine/mg protein and for ML-II cells were 0.2 and 0.8 nmol cystine/mg protein.

by chloroquine. Furthermore, the cystine in ML-II cells is depleted by cysteamine, albeit less completely than in cystinotic cells (27). The major difference in cystine accumulation between these cell types is that many weeks are required for the accumulation to occur in ML-II fibroblasts whereas the cystine content of cystinotic fibroblasts is always elevated. Since it is difficult to imagine how a specific defect in the targeting of the cystine transport protein to the lysosome could require this time to express itself, we do not favor this possibility.

A more plausible explanation is that one or more lysosomal enzymes which are required for normal lysosomal membrane integrity are defective in these ML-II fibroblasts. This would lead to an altered lysosomal membrane which could retard cystine efflux. Specific vectorial transport of a molecule across a membrane may require, in addition to a transport protein, properly charged and oriented functional groups at or near the binding site, mobility within the membrane, and a pH or potential gradient (28). One or more of these conditions may be abnormal in ML-II lysosomes. With regard to charged groups which might affect substrate binding or membrane potential, the lack of neuraminidase activity in ML-II cells (24, 25) leads to an increase in the quantity of sialic acid-containing residues and hence of negative charges. Our observation (Fig. 1) that the position of the lysosome peak in the pattern from free-flow electrophoresis is somewhat shifted toward the anode with respect to the normal and cystinotic lysosome peaks is consistent with a more negative surface charge. Further evidence in support of an altered membrane structure has been provided by Sly *et al.* (29) who showed that after passage through ML-II cells, Sindbis virus became unstable to freezing and hypersensitive to inactivation by Triton X-100. The sensitivity to freezing was attributed to viral envelope components derived from the host ML-II cell plasma membrane which carried an excess of sialic acid residues (30). The incomplete cysteamine-mediated depletion of ML-II cystine might be explained by a difference in membrane charge or permeability. Cysteamine has been shown to deplete lysosomal cystine by forming cysteamine-cysteine mixed disulfide (31) and then exiting the lysosome either by diffusion through the membrane or by utilizing the lysine transport system (32). A more negative charge might retard the positively charged disulfide or alter its binding for transport. A similar argument could be invoked to explain the reduction of the transport of cystine itself.

Another possible explanation for the cystine accumulation that occurs after prolonged culture of ML-II fibroblasts is that cystine egress is blocked by the presence of undigested inclusions.

Table 3. *Cystine reaccumulation after cysteamine depletion**

	Cystinosis (%)	ML-II (%)
Medium alone	70	110
50 μM chloroquine	29	45
30 mM cysteine glutathione disulfide	300	172

* Cells were depleted of cystine with a 1 h exposure to 5 mM cysteamine. They were then rinsed in PBS, placed in complete culture medium, and assayed for cystine content 24 h later. Values represent the amount of cystine accumulated after 24 h compared to the undepleted controls. Each value is the mean of two ML-II cell types and two cystinotic cell types.

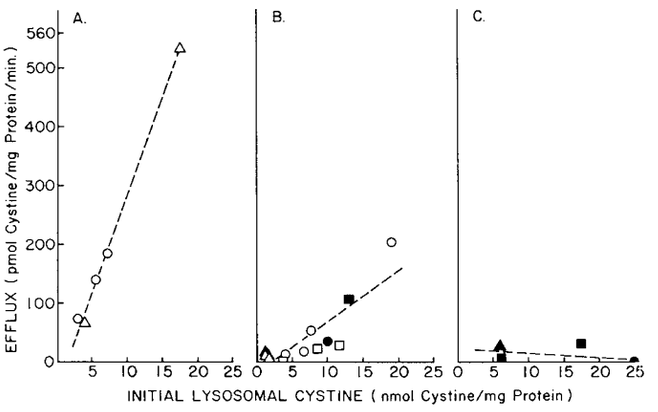

Fig. 3. Cystine loss from granular fractions of normal, ML-II, and cystinotic fibroblasts. Fibroblasts were harvested by scraping from confluent roller bottles, the granular fraction prepared, efflux in the presence of 2 mM ATP, 5 mM $MgCl_2$ performed as described in "Methods," and the rate plotted against lysosomal cystine content at the initial time point. The *dotted line* represents the slope generated by linear regression from all data points regardless of cell line. *A*, normal fibroblasts from two donors (○, △) were treated as above, but following scraping, the cells were suspended in cystine-free medium without serum and incubated 15 min at 37° C in 0.5 or 1.0 mM cystine dimethyl ester. *B*, ML-II fibroblasts from three cell lines, M.D. (△), L.T. (○), L.M. (□) were treated as above, but without a step for loading with cystine dimethyl ester. *Filled symbols* represent experiments in which a mock-loading step was included. *C*, cystinotic fibroblasts from three cell lines were treated as above including a mock-loading step. The two points at low initial cystine content were obtained by incubation 14 h in cystine-free medium with dialyzed fetal bovine serum before harvesting.

This would be consistent with the time course of the appearance of both the inclusions and cystine in these cells. In addition, this concept might explain the 4-h lag which is observed in cystine reaccumulation which ML-II cells demonstrate following cysteamine depletion of cystine. The undigested inclusions may provide thiol groups available to form disulfide linkages with either cysteine or cysteamine. As cystine and protein-bound cysteine is released from the lysosome by the formation of cysteine-cysteamine, the inclusions may form disulfides with cysteamine. When cysteamine is subsequently replaced by complete medium, the accumulation of free cystine is not detected until residual disulfide-bound cysteamine is displaced by exchange with cystine and exits the lysosome. In addition, the extensive membrane layers observed in residual, secondary lysosomes from ML-II fibroblasts (24) may sequester part of the cystine between the layers so that it can neither efflux nor react with cysteamine. Furthermore, if the cystine transport protein is retained in these layers, it may bind cystine but be unable to bring about translocation if no pH or potential gradient exists between the inner layers. This would explain our observation that ML-II cells are not depleted of cystine as completely as cystinotic cells and that cystine efflux is slow. The inclusions may also block access of cystine to the transporter sites by nonspecific binding.

The variance both in accumulation of cystine and in efflux capacity seen within the three ML-II cell lines studied may be the result of differences in any of the aspects discussed above. Since the ML-II condition arises from more than one mutation as revealed by complementation studies (33, 34), such differences may arise because the phenotypes determine membrane alterations, lysosomal enzyme levels, and amounts of unhydrolyzed inclusions. These, in turn, may affect the transport of cystine.

The failure of Steinherz *et al.* (1) to find impairment in cystine clearance from ML-II fibroblasts may be caused by the methodology they used. Their cultured ML-II fibroblasts were loaded with ^{35}S-cystine dimethyl ester followed by a 60-min chase. These studies show comparable ratios of ^{35}S-cysteine-N-ethylmaleimide to ^{35}S-cysteine following the chase period for both normal and ML-II cells. The ML-II cells, however, failed to hydrolyze completely the cystine dimethyl ester. In our preliminary studies we also found that cystine dimethyl ester was not completely hydrolyzed by ML-II fibroblasts. Therefore, we utilized a method that did not require use of this ester. Another possible reason that Steinherz *et al.* (1) did not observe defective lysosomal cystine transport in ML-II fibroblasts is that their cells may have been at an early stage of growth at which cystine accumulation was not yet evident.

The reason for the exclusively *in vitro* accumulation of cystine by ML-II cells remains uncertain. Upon reaching confluency, more protein is degraded via the lysosomal pathway in both normal and ML-II fibroblasts (35). In addition, the accumulation of undigested inclusions is progressive. These facts may explain why increased cystine is only observed in extended tissue culture.

In conclusion, we consider the most likely explanation of our observations is that altered membrane structure, the presence of undigested inclusions, and the increase in the lysosomal pathway of protein degradation after confluence all contribute to the accumulation of cystine in ML-II cell lysosomes.

Acknowledgments. The authors thank Hildegard Kern, Karen F. Clark, and Michelle Cass for their technical assistance and Cynthia Knowles for aid with the manuscript.

REFERENCES

1. Steinherz R, Makov N, Narinsky R, Meidan B, Kohn G 1984 Comparative study of cystine clearance in cystinotic and I-cell fibroblasts upon exposure to cystine dimethyl ester. Enzyme 32:126–130
2. Schneider JA, Rosenbloom FM, Bradley KH, Seegmiller JE 1967 Increased free-cystine content of fibroblasts cultured from patients with cystinosis. Biochem Biophys Res Commun 29:527–531
3. Schneider, JA, Schulman, JD 1983 Cystinosis. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The Metabolic Basis of Inherited Disease, 5th ed. McGraw-Hill, New York, pp 1844–1866
4. Jonas AJ, Greene AA, Smith ML, Schneider JA 1982 Cystine accumulation and loss in normal, heterozygous, and cystinotic fibroblasts. Proc Natl Acad Sci USA 79:4442–4445
5. Steinherz R, Tietze F, Gahl WA, Triche T, Chiang H, Modesti A, Schulman JD 1982 Cystine accumulation and clearance by normal and cystinotic leucocytes exposed to cystine dimethyl ester. Proc Natl Acad Sci USA 79:4446–4450
6. Gahl WA, Tietze F, Bashan N, Steinherz R, Schulman JD 1982 Defective cystine exodus from isolated lysosome-rich fractions of cystinotic leucocytes. J Biol Chem 257:9570–9575
7. Gahl WA, Bashan N, Tietze F, Bernardini I, Schulman JD 1982 Cystine transport is defective in isolated leucocyte lysosomes from patients with cystinosis. Science 217:1263–1265
8. Jonas AJ, Smith ML, Schneider JA 1982 ATP-dependent lysosomal cystine efflux is defective in cystinosis. J Biol Chem 257:13185–13188
9. Jonas AJ, Smith ML, Allison WS, Laikind PK, Greene AA, Schneider JA 1983 Proton-translocating ATPase and lysosomal cystine transport. J Biol Chem 258:11727–11730
10. Gahl WA, Tietze F, Bashan N, Bernardini I, Raiford D, Schulman JD 1983 Characteristics of cystine counter-transport in normal and cystinotic lysosome-rich leucocyte granular fractions. Biochem J 216:393–400
11. Tietze F, Butler JD 1979 Elevated cystine levels in cultured skin fibroblasts from patients with I-cell disease. Pediatr Res 13:1350–1355
12. Coon HG, Weiss MC 1969 A quantitative comparison of formation of spontaneous and virus-produced viable hybrids. Proc Natl Acad Sci USA 62:852–859
13. Eriksson B, Eriksson SA 1967 Synthesis and characterization of the L-cysteine-glutathione mixed disulfide. Acta Chim Scand 21:1304–1312
14. Oshima RG, Willis RC, Furlong CE, Schneider JA 1974 Binding assays for amino acids: The utilization of a cystine binding protein from Escherichia coli for the determination of acid-soluble cystine in small physiological samples. J Biol Chem 249:6033–6039
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
16. Dulbecco R, Vogt M 1954 Plaque formation and isolation of pure lines with poliomyelitis viruses. J Exp Med 99:167–182
17. Harms E, Kern H, Schneider JA 1980 Human lysosomes can be purified from diploid skin fibroblasts by free-flow electrophoresis. Proc Natl Acad Sci USA 77:6139–6143
18. Bradford, MM 1976 A rapid and sensitive method for the quantitation of microgram, quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
19. Sedmak JJ, Grossberg SE 1977 A rapid sensitive and versatile assay for protein using Coomassie brilliant blue G250. Anal Biochem 79:544–552
20. Gianetto R, deDuve C 1955 Comparative study of the binding of acid phosphatase, β-glucuronidase and cathepsin by rat-liver particles. Biochem J 59:433–438
21. Leroy JG, DeMars RI 1967 Mutant enzymatic and cytological phenotypes in cultured human fibroblasts,. Science 157:804–806
22. Pennington RJ 1961 Mitochondrial succinate-tetrazolium reductase and adenosine triphosphatase. Biochem J 80:649–654
23. Schneider JA, Verroust FM, Kroll WA, Garvin AJ, Horger EO, Wong VG, Spear GS, Jacobsen C, Pellett OL, Becker FLA 1974 Prenatal diagnosis of cystinosis. N Engl J Med 290:878–882
24. Miller AL, Kress BC, Stein R, Kinnon C, Kern H, Schneider JA, Harms E 1981 Properties of N-acetyl β-D-hexosaminidase from isolated normal and I-cell lysosomes. J Biol Chem 256:9352–9362
25. Thomas GH, Tiller GE, Reynolds LW, Miller CS, Base JW 1976 Increased levels of sialic acid associated with a sialidase deficiency in I-cell disease (mucolipidosis II) fibroblasts. Biochem Biophys Res Commun 71:188–195
26. Thoene JG, Oshima RG, Ritchie DG, Schneider JA 1977 Cystinotic fibroblasts accumulate cystine from intracellular protein degradation. Proc Natl Acad Sci USA 74:4505–4507
27. Thoene JG, Oshima RG, Crawhall JC, Olson DL, Schneider JA 1976 Intracellular cystine depletion by aminothiols in vitro and in vivo. J Clin Invest 58:180–189
28. Christensen HN 1975 Biological Transport, 2nd ed. WA Benjamin, Inc, London
29. Sly WS, Lagwinska E, Schlesinger S 1976 Enveloped virus acquired membrane defect when passaged in fibroblasts from I-cell disease patients. Proc Natl Acad Sci USA 73:2443–2447
30. Schlesinger S, Sly WS, Shulze IT 1978 Effects of neuraminidase on the phenotype of Sindbis virus grown in fibroblasts obtained from patients with I-cell disease. Virology 89:409–417
31. Butler J deB, Zatz M 1984 Pantethine and cysteamine deplete cystine from cystinotic fibroblasts via efflux of cysteamine-cysteine mixed disulfide. J Clin Invest 74:411–416
32. Pisoni RL, Thoene JG, Christensen HN 1985 Detection and characterization of carrier-mediated cationic amino acid transport in lysosomes of normal and cystinotic human fibroblasts. Role in therapeutic cystine removal? J Biol Chem 260:4791–4798
33. Shows TB, Mueller OT, Honey NK, Wright CE, Miller AL 1982 Genetic heterogeneity of I-cell disease is demonstrated by complementation of lysosomal enzyme processing mutants. Am J Med Genet 12:343–353
34. Mueller OT, Honey NK, Little LE, Miller AL 1983 Mucolipidosis II and III. The genetic relationships between two disorders of lysosomal enzyme biosynthesis. J Clin Invest 72:1016–1023
35. Cockle SM and Dean RT 1984 An abnormality in intracellular protein degradation in fibroblasts from patients with I-cell disease. Clin Chim Acta 140:257–265