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Demonstration of the Heterozygous State for I-Cell Disease and Pseudo-Hurler Polydystrophy by Assay of *N*-Acetylglucosaminylphosphotransferase in White Blood Cells and Fibroblasts

AJIT VARKI,[1] MARC L. REITMAN,[1] ANN VANNIER,[1] STUART KORNFELD,[1] JEFFREY H. GRUBB,[2] AND WILLIAM S. SLY[2]

SUMMARY

The biochemical abnormalities of I-cell disease (mucolipidosis II) and pseudo-Hurler polydystrophy (mucolipidosis III) can be explained by a deficiency of the enzyme UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase. We demonstrate here that obligate heterozygotes for these autosomal recessive diseases have intermediate levels of this enzymatic activity in homogenates of peripheral blood white cells and in extracts from cultured fibroblasts. This finding provides further evidence that the enzyme deficiency is the primary genetic defect in these diseases. In addition, the previous observation that obligate heterozygotes for mucolipidosis III have elevations of total serum β-hexosaminidase outside the range of normal was confirmed. In studies of three pedigrees of patients with mucolipidosis III, these techniques were used to score individuals at risk for the carrier state.

INTRODUCTION

I-cell disease (mucolipidosis II) and pseudo-Hurler polydystrophy (mucolipidosis III) are autosomal recessive lysosomal storage disorders characterized by skeletal abnormalities, psychomotor retardation, and coarse facial features. I-cell disease is clinically the more severe of the two, with an earlier onset, and a more rapidly

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[1] Departments of Internal Medicine and Biological Chemistry, Washington University School of Medicine, St. Louis, Mo.

[2] The Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, Division of Medical Genetics, St. Louis Children's Hospital, St. Louis, Mo.

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fatal course. One of the most striking biochemical features of these diseases is the finding of markedly elevated levels of many lysosomal enzymes in the serum. Cultured fibroblasts from these patients show decreased intracellular activities of the same lysosomal enzymes and have characteristic inclusion bodies [1].

We [2] and Hasilik et al. [3] showed that these biochemical abnormalities can be explained by a deficiency of UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (*N*-acetylglucosaminylphosphotransferase). The lack of this enzyme precludes the generation of the common phosphomannosyl recognition marker [3–18] of lysosomal enzymes. As a consequence, newly synthesized lysosomal enzymes are not targeted to the lysosomes, but are secreted into the extracellular space instead. We have also shown that the difference in clinical severity between the two syndromes may be explained by the relative degree of deficiency of the *N*-acetylglucosaminylphosphotransferase [19].

We demonstrate here the heterozygous state for mucolipidosis II (ML II) and mucolipidosis III (ML III) by assay of *N*-acetylglucosaminylphosphotransferase in peripheral blood white cells and in cultured fibroblasts. We also evaluate the use of this enzyme measurement along with measurements of total serum β-hexosaminidase [20–22] in screening for the heterozygous state for mucolipidosis III.

MATERIALS AND METHODS

Materials

Materials were obtained from the following sources: 3a70 scintillation cocktail, Triton X-100, and the 4-methylumbelliferyl enzyme substrates (Research Products, Mount Prospect, Ill.); Concanavalin A-Sepharose (10 mg/ml) and Ficoll-Paque (Pharmacia, Piscataway, N.J.); [γ-^{32}P]ATP (2,000 Ci/mmol) and UDP-[6-^{3}H]GlcNAc (6.6 Ci/mmol) (New England Nuclear, Boston, Mass.); α-minimum essential medium (Flow Laboratories, Rockville, Md.); fetal bovine serum, penicillin, and streptomycin (Gibco, Grand Island, N.Y.); Panheprin (Heparin USP) (Abbott, North Chicago, Ill.); QAE-Sephadex (Q-25-120), α-methyl-D-mannoside, UDP-GlcNAc, ATP, clinical grade dextran (mol. wt. 200,000–275,000), *p*-nitrocatechol sulfate, and other reagents (Sigma, St. Louis, Mo.). [β-^{32}P]UDP-GlcNAc was prepared as described [13].

Preparation of White Blood Cell Homogenates

Peripheral venous blood was drawn into heparinized syringes (275 USP U heparin/10 ml blood). Ten ml of heparinized blood was mixed with 30 ml of 3% Dextran in 0.9% NaCl (containing 15 USP U/ml heparin) and allowed to sediment in an inverted 60-ml syringe for 40 min. The dextran supernatant was then pushed out of the syringe through a 20-gauge needle bent at a 45° angle into a cold polyethylene centrifuge tube. After centrifugation at 270 *g* for 8 min at 4°C, the supernatant was poured off and the pellet resuspended in 5 ml ice cold distilled water to lyse any remaining red blood cells (RBCs). After exactly 45 seconds, 5 ml 1.8% NaCl was added to make the solution isotonic. The white blood cells (WBCs)

were pelleted by centrifugation at 480 *g* for 8 min at 4° C, and washed once with 5 ml 0.9% NaCl. The pellet was resuspended in 0.5 ml distilled water and homogenized in a Duall-type glass homogenizer (size 20) using 10 strokes with a ground glass pestle.

WBC Fractionation

Twenty-five ml of heparinized blood was collected and a 10-ml aliquot used to isolate total WBCs as described above. To another 10 ml of the heparinized blood, 10 ml of buffered salt solution (BSS) (0.01% glucose, 0.005 mM $CaCl_2$, 0.098 mM $MgCl_2$, 0.54 mM KCl, 14.5 mM Tris, pH 7.6, and 126 mM NaCl) was added in a siliconized tube. After mixing with a siliconized Pasteur pipet, 10 ml of this BSS/blood mixture was layered over 7 ml of Ficoll-Paque in a 30-ml siliconized Corex centrifuge tube and centrifuged at 400 *g* for 35 min at room temperature. The lymphocyte/monocyte layer was collected from the interface, mixed with 3 vol of BSS, and centrifuged at 100 *g* for 10 min at room temperature. The pellet was resuspended in saline. The granulocyte/RBC pellet from the Ficoll-Paque separation was resuspended in BSS and the granulocytes separated by the dextran sedimentation procedure used to isolate total WBCs. The granulocyte pellet was resuspended in 0.9% NaCl. Sonicated extracts were made from each WBC fraction using a Bronwill Biosonik III sonicator (two 12-sec bursts, miniprobe on setting 30).

Analytical Methods

Protein was measured by the method of Lowry et al. [23]. Lysosomal enzymes were measured on an aliquot of the WBC homogenate that had been frozen at −70° C and thawed to release latent activity. Fluorometric enzyme assays measured release of 4-methylumbelliferone from the appropriate glycoside [2]. Synthetic substrate concentrations were 10 mM (in 0.1 M sodium acetate, pH 4.8) for β-glucuronidase; 1 mM (in 0.08 M sodium phosphate-citrate, pH 4.4) for β-galactosidase; 1 mM (in 0.05 M sodium citrate, pH 4.0) for α-mannosidase; and 0.96 mM (in 0.1 M sodium phosphate-citrate, pH 4.5) for α-fucosidase. In all of these assays, 25 μl of WBC extract (11–32 μg protein) was added to 100 μl of substrate and the mixture incubated at 37° C for 30–60 min. β-Hexosaminidase was assayed using 5 mM 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside (in 0.02 M sodium citrate-phosphate, pH 4.4) as substrate. Fifty μl of diluted serum (1:10 in 0.02 M sodium citrate-phosphate, pH 4.4) or 2–6 μg of WBC protein was mixed with 100 μl of substrate and incubated for 30 min at 37° C. All of the above reactions were stopped by the addition of 1.8 ml glycine-carbonate buffer, pH 10.3, and the 4-methylumbelliferone product measured fluorometrically [24]. Arylsulfatase A was assayed by the method of Baum et al. [25], using *p*-nitrocatechol sulfate as the substrate. One U of each of the above is defined as the amount of enzyme that catalyzes the release of 1 nmol of product per hr.

Cells

Fibroblast cultures were obtained from the Human Genetic Mutant Cell repository, Camden, N.J. (see legend to fig. 4 for a detailed list), except for the following subjects: Y. F., S. F., P. D., and E. D. (from Dr. Arnold Miller, University of California at San Diego); subject A. T. (from Dr. George Thomas, Johns Hopkins University); subjects C. E. and J. E. (patients of Dr. William Sly), and the normal fibroblasts (from Dr. Eugene Bauer, Washington University). The conditions for growth, maintenance, and passage of cells were exactly as previously described [2].

Assay of N-*Acetylglucosaminylphosphotransferase*

In WBC. The assay measures the transfer of GlcNAc 1-[^{32}P]-phosphate from [β-^{32}P]UDP-GlcNAc to the 6-hydroxyl position of the acceptor α-methylmannoside, and has been described in detail [16, 19]. The final reaction mixture contained 150 μg of WBC protein, 100 μg of bovine serum albumin, 100 mM α-methylmannoside, 10 mM $MnCl_2$, 10 mM $MgCl_2$, 0.25 mM Dithiothreitol, 5 mM ATP, 0.75% Triton X-100 (w/v), and 150 μM [β-^{32}P]UDP-GlcNAc ($3–4 \times 10^5$ cpm) in a 50-μl reaction volume. The reactions were carried out for 30 min at 37°C and quenched by addition of 50 μl 40 mM EDTA. The product (GlcNAc-[^{32}P]P-α-methylmannoside) was isolated and quantitated using columns of QAE-Sephadex as described [16, 19]. The structure and purity of the product was proven by paper chromatography before and after acid hydrolysis [16] (data not shown). Under these conditions, the assay was linear with time and protein concentration. Simultaneous blanks were set up by adding the EDTA at the start of the reaction. One U of activity is defined as the transfer of 1 pmol GlcNAc 1-P/hr. The activity in the homogenate was stable on ice for over 12 hrs, but it was necessary to disperse any clumps formed thoroughly (by sonication) to ensure reproducibility. This assay can also be carried out using UDP-[6-^{3}H]GlcNAc as the tracer, but the background is higher (in this case the blanks should be set up by substituting 100 mM α-methylgalactoside for the α-methylmannoside).

In fibroblasts. The preparation of fibroblast homogenates, and the assay of *N*-acetylglucosaminylphosphotransferase using either exogenous [19] or endogenous [2] acceptors has been described. All assays were carried out on confluent cultures 7–10 days after passage.

RESULTS

Assay of N-*Acetylglucosaminylphosphotransferase in Fibroblasts*

As reported elsewhere, fibroblast extracts from patients with ML II and ML III have markedly depressed values of *N*-acetylglucosaminylphosphotransferase [2, 19]. To determine whether obligate heterozygotes have intermediate levels of this enzyme (as one would predict if the *N*-acetylglucosaminylphosphotransferase deficiency were the primary defect in these disorders), we examined the enzyme level in fibroblast extracts from parents of patients with ML II and ML III. From the data presented in figure 1, it is clear that the levels of *N*-acetylglucosaminylphosphotransferase activity in fibroblasts from obligate heterozygotes are significantly

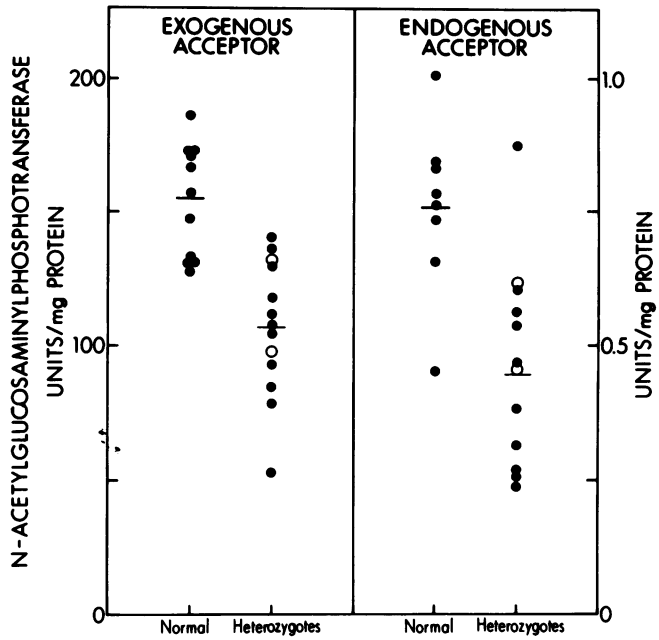

FIG. 1.—*N*-acetylglucosaminylphosphotransferase activity in fibroblast cultures, using exogenous (α-methylmannoside) and endogenous (glycoprotein) acceptors. *Each dot* represents the mean of two sets of duplicate determinations on fibroblast cultures from separate individuals. Heterozygotes are parents of patients with ML II except for the *open circles* that represent parents of patient with ML III. Individual heterozygotes are: (*from top to bottom*) exogenous acceptor assay—GM 1590, GM 1589, C. E., GM 80, GM 2046, Y. F., GM 81, A. T., J. E., S. F., GM 2047, P. D., and E. D.: endogenous acceptor assay—Y. F., C. E., S. F., GM 1589, GM 2046, P. D., J. E., GM 1590, GM 80, E. D.; GM 2047, and A. T. *Horizontal bars* indicate mean values for each group. Levels of activity in fibroblasts from homozygotes have been reported [2, 19] (exogenous acceptor assay: ML III 3.0–39.4 U/mg; ML II $<$0.4–2.2 U/mg; endogenous acceptor assay: ML III $<$ 0.02–0.27 U/mg; ML II $<$ 0.2 U/mg).

lower than those in normal fibroblasts with both exogenous (α-methylmannoside) or endogenous (glycoprotein) assays, although there is some overlap between the groups. We found that the assay using either acceptor was significantly affected by the stage of growth of the fibroblasts. To control for this variable, all studies were carried out on newly confluent cultures, between 7 and 10 days after passage.

Assay of N-*Acetylglucosaminylphosphotransferase in WBC Homogenates*

The levels of *N*-acetylglucosaminylphosphotransferase in WBC homogenates from patients with ML III, their parents, other family members, and normal controls are shown in figure 2. The patients have extremely low levels of enzyme activity. Their parents have significantly decreased levels, as would be expected for obligate heterozygotes if the *N*-acetylglucosaminylphosphotransferase deficiency is the primary defect in ML III. The other members of the families have the expected distribution for individuals at risk, with some being in the normal and others in the heterozygote range.

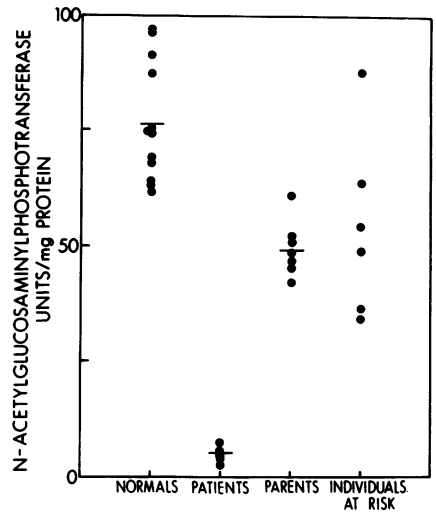

FIG. 2.—*N*-acetylglucosaminylphosphotransferase activity in leukocyte extracts. *Each dot* represents the mean of two separate determinations (done in duplicate) on each individual. Patients with ML III, parents, and individuals at risk are from the three pedigrees shown in figure 4 and table 1. Data from one additional patient with ML III and his mother are also included. *Horizontal bars* indicate mean values of each group.

To study further the distribution of the *N*-acetylglucosaminylphosphotransferase among the different white cell types, we separated normal WBCs into granulocytic and mononuclear cell fractions and analyzed each separately. The results of a typical experiment are as follows: total WBCs, 95.9 U/mg protein; mononuclear cell fraction, 223 U/mg protein; and granulocytic fraction, 10.4 U/mg protein. Thus, the mononuclear cell fraction has a much higher specific activity of the *N*-acetylglucosaminylphosphotransferase than the granulocytic fraction.

Total Serum β-Hexosaminidase Levels

Serum was prepared from the same blood samples for determination of total β-hexosaminidase levels. As expected, the patients with ML III have markedly elevated levels (see table 1). As shown in figure 3, all of the obligate heterozygotes (parents) have moderately increased values while the other family members at risk have either normal or moderately elevated levels.

Detailed Analysis of Pedigrees and Scoring of the Carrier State

The pedigrees of three complete families of patients with ML III that were studied are shown in figure 4. Table 1 shows the actual levels of WBC *N*-acetylglucosaminylphosphotransferase and total serum hexosaminidase in each individual of these pedigrees. When these two parameters were used to score individuals at risk for the carrier state, there were three definite carriers (elevated serum β-hexosaminidase, decreased transferase—E-II-1, F-II-3, and D-I-3), one definite noncarrier (normal β-hexosaminidase and transferase—E-II-3), and two possible

carriers (elevated serum β-hexosaminidase alone—D-II-2; decreased transferase alone—E-II-4). In one of these individuals (D-II-2), the transferase level was at the lower limits of the normal range.

Lysosomal Enzyme Levels in the WBC Homogenates

Since previous studies have suggested tissue-specific involvement in I-cell disease [1, 26], with the WBC being spared [27], we also examined the levels of lysosomal enzymes in the total WBC homogenates. The results are shown in table 2. It can be seen that the levels of six lysosomal enzymes are essentially normal in the WBCs of both heterozygotes and homozygotes for ML III (except for β-galactosidase which is ~60% of normal in the homozygotes). Furthermore, when WBCs were fractionated into mononuclear and granulocytic fractions, each fraction also had lysosomal enzyme levels similar to the normal (data not shown). Thus, in spite of the deficiency of the *N*-acetylglucosaminylphosphotransferase, the WBCs are capable of attaining normal intracellular levels of lysosomal enzymes.

TABLE 1

LEUKOCYTE *N*-ACETYLGLUCOSAMINYLPHOSPHOTRANSFERASE AND TOTAL SERUM β-HEXOSAMINIDASE LEVELS IN ML III FAMILIES

	Leukocyte *N*-acetylglucosaminylphosphotransferase (U/mg)	Total serum β-hexosaminidase (U/ml)
Family E:		
I-1	51.1	3,164
I-2	42.7	2,344
II-1	34.1	2,764
II-2*	2.5	19,840
II-3	87.9	1,420
II-4	54.4	1,600
Family F:		
I-1	61.4	3,484
I-2	45.4	2,180
II-1*	7.6	20,720
II-2*	4.9	19,320
II-3	49.3	2,820
Family D:		
I-1	49.9	2,104
I-2	50.4	2,544
I-3	36.8	3,380
II-1*	5.7	17,920
II-2	64.0	1,900
Normal controls:		
Mean (±2 SD)	76.5 ± 12.5 (no. = 12)	1270 ± 298 (no. = 200)
Range	62.1–97.0	684–2412
Obligate heterozygotes†:		
Mean (±2 SD)	49.7 ± 6 (no. = 7)	2771 ± 625 (no. = 7)
Range	42.7–61.4	2104–3580

* Patients with ML III.

† Includes one additional parent (obligate heterozygote) of a patient with ML III, not shown in this table.

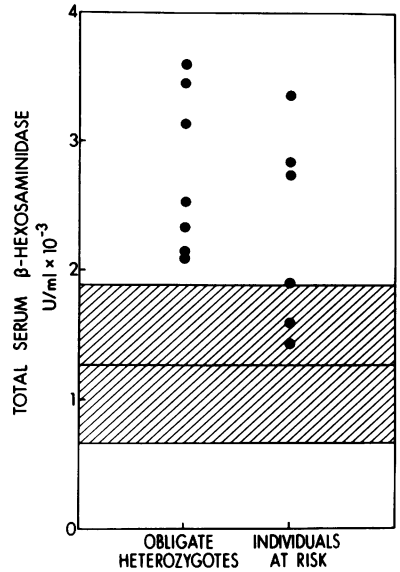

FIG. 3.—Total serum β-hexosaminidase activity in obligate heterozygotes (parents of patients with ML III) and individual at risk for heterozygosity. Individuals (*each represented by one dot*) are same as those shown in figure 2. *Hatched area* shows limits of mean ± 2 SD for 200 normal controls.

DISCUSSION

There is now considerable evidence indicating that the common phosphomannosyl recognition marker of lysosomal enzymes is involved in their targeting to the lysosomes [4–12]. The first step in the generation of this recognition marker involves the phosphorylation of the high mannose-type oligosaccharides of the lysosomal enzymes. This occurs by transfer of *N*-acetylglucosamine 1-phosphate to the 6 hydroxyl of mannose residues from the nucleotide sugar donor UDP-*N*-acetylglucosamine [3, 13, 16]. Subsequently, the outer *N*-acetylglucosamine residue is removed by a second enzyme [14, 15, 18]. In I-cell disease (ML II) and pseudo-Hurler polydystrophy (ML III), deficiency of the *N*-acetylglucosaminylphosphotransferase responsible for the first step results in a failure to generate the phosphorylated recognition marker. As a consequence, the newly synthesized lysosomal enzymes are secreted into the medium of fibroblast cultures instead of being targeted to the lysosomes.

Here we demonstrate that freshly collected peripheral blood white cells and cultured fibroblasts from parents (obligate heterozygotes) of patients with these diseases have intermediate levels of *N*-acetylglucosaminylphosphotransferase activity. This provides further evidence that the deficiency of this enzyme is the primary genetic defect in these diseases. We have also recently used this enzyme measurement to aid in defining the mechanism of an unusual case of mosaicism in a 46,XY male with ML II [28].

However, it should be noted that when using the α-methylmannoside acceptor assay, a normal level of *N*-acetylglucosaminylphosphotransferase activity does not necessarily imply the absence of heterozygosity or even of the disease itself. In other studies, we found one subgroup of ML III patients that have normal levels of *N*-acetylglucosaminylphosphotransferase activity with this substrate [19]. In this variant form of ML III, the *N*-acetylglucosaminylphosphotransferase fails to phosphorylate lysosomal enzymes in spite of normal catalytic activity toward the exogenous monosaccharide acceptor. Thus, the assay utilizing either the endogenous acceptor glycoproteins or exogenously added lysosomal enzymes as acceptors gives very low values, even though the assay with α-methylmannoside gives results in the normal range. Although we have not had the opportunity to study the parents of these patients, it is reasonable to assume that the obligate heterozygotes for this form of ML III would have normal activity if their white cells or fibroblasts were assayed using the α-methylmannoside method.

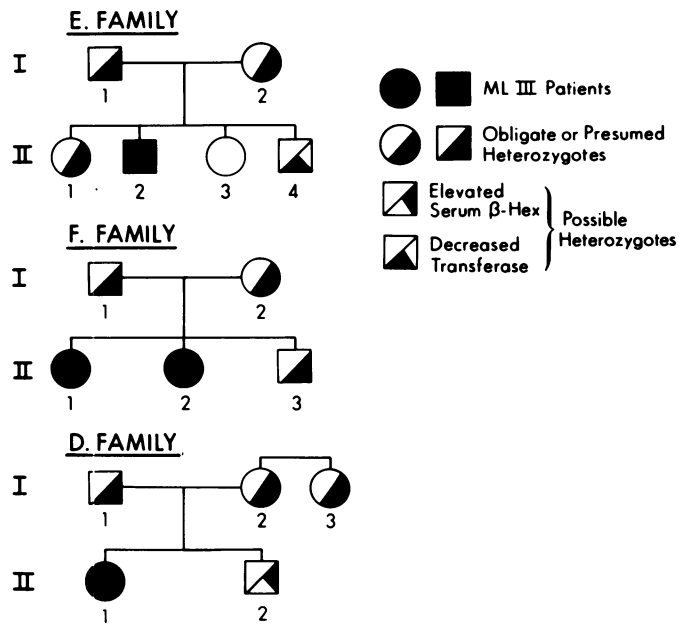

FIG. 4.—Pedigrees of ML III families. Table 1 summarizes leukocyte *N*-acetylglucosaminylphosphotransferase and total serum hexosaminidase levels in members of these pedigrees and controls. Pedigrees contain six obligate heterozygotes (parents of probands) and six persons at risk of being heterozygotes for ML III. Using two criteria, reduction of *N*-acetylglucosaminylphosphotransferase to within 2 SD above the mean for obligate heterozygotes, and elevation of total serum β-hexosaminidase by 2 SD above the mean for normal controls, three of the six at risk were scored as carriers, one as a noncarrier, and two were scored as possible carriers, satisfying only one of the two criteria. Proband from family E was previously reported [27] and fibroblasts from this patient are GM 3685. The two affected patients in family F have moderate mental retardation and severe joint disability. Their fibroblasts are available as GM 2558 and GM 2559. Proband from family D is very mildly retarded, and has very minimal joint disability.

TABLE 2

LYSOSOMAL ENZYME LEVELS IN LEUKOCYTE HOMOGENATES

ENZYME	ACTIVITY (NMOL/MG/HR) Controls (no. = 14)	Obligate heterozygotes (no. = 6)	Homozygotes (no. = 4)
β-Glucuronidase	357 ± 50	378 ± 50	354 ± 60
β-Galactosidase	242 ± 20	237 ± 20	151 ± 18
α-Fucosidase	59 ± 10	62 ± 15	60 ± 10
α-Mannosidase	149 ± 35	158 ± 50	168 ± 51
β-Hexosaminidase	1347 ± 244	1499 ± 242	1535 ± 291
Arylsulfatase A	183 ± 33	167 ± 18	175 ± 41

NOTE: All values are mean ± 2 SD.

It has previously been noted that obligate heterozygotes for these diseases may have slightly elevated levels of total serum β-hexosaminidase [20–22]. This proved to be the case in the families studied here. Although the levels of total serum β-hexosaminidase in heterozygotes were considerably lower than those found in the homozygotes, all of the seven obligate heterozygotes that we studied had levels that were above the range of normal. Thus, this measurement appears to be of value in screening for the heterozygous state. It is known that other factors (e.g., pregnancy, birth control pills) can also increase serum β-hexosaminidase levels [29]. It is not yet known what effect, if any, these or other factors such as age have on the *N*-acetylglucosaminylphosphotransferase assay. Nevertheless, using the two criteria of (1) decreased WBC *N*-acetylglucosaminylphosphotransferase, and (2) increased total serum β-hexosaminidase, three of the six individuals at risk had levels of both enzymes corresponding to those of obligate heterozygotes, and can therefore be scored as carriers. In two other persons at risk, only one of the two enzymes was clearly in the carrier range. It has also been noted that the fraction of serum β-hexosaminidase that is heat-labile can be increased in heterozygotes for ML II and ML III [20–22]. In the families studied here, however, this test proved less useful (data not shown). Others have reported [21, 22, 30] that heterozygotes for ML II and ML III could be identified by quantitation of the four β-hexosaminidase isozymes (B, I_1, I_2, and A) in plasma fractionated by chromatography on DEAE cellulose. Although this analysis may be too tedious to qualify as a routine screening procedure, it could be a valuable additional test for family members whose carrier status is not established unambiguously by simpler assays.

Previous studies have suggested that ML II and ML III are tissue-specific diseases, with certain cell types being spared [1, 20, 26, 27]. Our studies confirmed the previous observation that WBC extracts from patients with ML III have normal levels of most lysosomal enzymes [27]. However, the *N*-acetylglucosaminylphosphotransferase activity was markedly diminished in the same cell extracts. This would explain the prior observation of Glaser et al. [27] that, although WBC had normal levels of lysosomal enzymes in ML III, the enzymes extracted from these WBCs were not taken up by fibroblasts, suggesting that they lack the phospho-

mannosyl recognition marker. It is not clear how leukocyte enzymes that lack the phosphomannosyl enzyme marker are targeted to lysosomes. In preliminary experiments, we found that when peripheral blood WBCs from patients with ML III were separated into mononuclear and granulocytic fractions, each fraction also had levels of lysosomal enzymes that were not different from those of similar fractions obtained from normal controls. This would be in favor of the possibility that WBCs have alternate methods for intracellular targeting of newly synthesized acid hydrolases to lysosomes. Alternatively, these cells may be capable of taking up exogenous lysosomal enzymes that are present in high levels in the serum of these patients by a process that does not depend on the phosphomannosyl recognition marker. Our results do not clearly distinguish between these two possibilities.

The fact that the total serum β-hexosaminidase levels are significantly elevated in all of the obligate heterozygotes suggests that the reduction in enzyme level seen in heterozygotes, although insufficient to produce clinical abnormality, can result in failure to transfer enzymes to lysosomes, at least in some tissues. Thus, the level of *N*-acetylglucosaminylphosphotransferase in heterozygotes may be limiting in certain tissues and even half of the normal enzyme activity appears insufficient to insure completely normal targeting of the lysosomal enzymes in these tissues. This could also explain the previous finding that some patients with clinically obvious ML III can have levels of the *N*-acetylglucosaminylphosphotransferase as high as 25% of normal [19].

REFERENCES

1. McKusick VA, Neufeld EF, Kelly TE: Two disorders of lysosomal enzyme localization: mucolipidosis II (ML II, I-cell disease, inclusion cell disease and mucolipidosis III (ML III, pseudo-Hurler polydystrophy), in *The Metabolic Basis of Inherited Disease*, edited by Stanbury JB, Wyngaarden JB, Fredrickson DS, New York, McGraw-Hill, pp 1299–1302
2. Reitman ML, Varki A, Kornfeld S: Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in UDP-*N*-acetylglucosamine: glycoprotein *N*-acetylglucosaminylphosphotransferase activity. *J Clin Invest* 67:1574–1579, 1981
3. Hasilik A, Waheed A, von Figura K: Enzymatic phosphorylation of lysosomal enzymes in the presence of UDP-*N*-acetylglucosamine: absence of activity in I-cell fibroblasts. *Biochem Biophys Res Commun* 98:761–767, 1981
4. Hickman S, Neufeld EF: A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. *Biochem Biophys Res Commun* 49:992–999, 1972
5. Kaplan A, Achord DT, Sly WS: Phosphohexosyl components of a lysosomal enzyme are recognized by pinocytosis receptors on human fibroblasts. *Proc Natl Acad Sci USA* 74:2026–2030, 1977
6. Natowicz MR, Chi MMY, Lowry OH, Sly WS: Enzymatic identification of mannose 6-phosphate on the recognition marker for receptor-mediated pinocytosis of β-glucuronidase by human fibroblasts. *Proc Natl Acad Sci USA* 76:4322–4326, 1979
7. Distler J, Hieber V, Sahagian G, Schmickel R, Jourdian GW: Identification of mannose 6-phosphate in glycoproteins that inhibit the assimilation of β-galactosidase by fibroblasts. *Proc Natl Acad Sci USA* 76:4235–4239, 1979
8. Hasilik A, Neufeld EF: Biosynthesis of lysosomal enzymes in fibroblasts: phosphorylation of mannose residues. *J Biol Chem* 255:4946–4950, 1980
9. Bach G, Bargal R, Cantz M: I-cell disease: deficiency of extracellular hydrolase phosphorylation. *Biochem Biophys Res Commun* 91:976–981, 1979

10. TABAS I, KORNFELD S: Biosynthetic intermediates of β-glucuronidase contain high mannose oligosaccharides with blocked phosphate residues. *J Biol Chem* 255:6633–6639, 1980
11. HASILIK A, KLEIN U, WAHEED A, STRECKER G, VON FIGURA K: Phosphorylated oligosaccharides in lysosomal enzymes: identification of α-*N*-acetylglucosamine (1)phospho (6)mannose diester groups. *Proc Natl Acad Sci USA* 77:7074–7078, 1980
12. VARKI A, KORNFELD S: Structural studies of phosphorylated high mannose-type oligosaccharides. *J. Biol Chem* 255:10847–10858, 1980
13. REITMAN ML, KORNFELD S: UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosamine-1-phosphotransferase: proposed enzyme for the phosphorylation of the high mannose oligosaccharide units of lysosomal enzymes. *J Biol Chem* 256:4275–4281, 1981
14. VARKI A, KORNFELD S: Identification of a rat liver α-*N*-acetylglucosaminyl phosphodiesterase capable of removing "blocking" α-*N*-acetylglucosamine residues from phosphorylated high mannose oligosaccharides of lysosomal enzymes. *J Biol Chem* 255: 8389–8401, 1980
15. VARKI A, KORNFELD S: Purification and characterization of rat liver α-*N*-acetylglucosaminyl phosphodiesterase. *J Biol Chem* 256:9937–9943, 1981
16. REITMAN ML, KORNFELD S: Lysosomal enzyme targeting: *N*-acetylglucosaminylphosphotransferase selectively phosphorylates native lysosomal enzymes. *J Biol Chem* 256:11977–11980, 1981
17. WAHEED A, POHLMANN R, HASILIK A, VON FIGURA K: Subcellular location of two enzymes involved in the synthesis of phosphorylated recognition markers in lysosomal enzymes. *J Biol Chem* 256:4150–4152, 1981
18. WAHEED A, HASILIK A, VON FIGURA K: Processing of the phosphorylated recognition marker in lysosomal enzymes: characterization and partial purification of a microsomal α-*N*-acetylglucosaminyl phosphodiesterase. *J Biol Chem* 256:5717–5721, 1981
19. VARKI A, REITMAN ML, KORNFELD S: Identification of a variant of mucolipidosis III (pseudo-Hurler polydystrophy): a catalytically active *N*-acetylglucosaminylphosphotransferase that fails to phosphorylate lysosomal enzymes. *Proc Natl Acad Sci USA* 78:7773–7777, 1981
20. KELLY TE, THOMAS GH, TAYLOR HA JR, ET AL.: Mucolipidosis III (pseudo-Hurler polydystrophy): clinical and laboratory studies in a series of 12 patients. *Johns Hopkins Med J* 137:156–175, 1975
21. LEROY JG, VAN ELSEN AF: I-cell disease (mucolipidosis II)—serum hydrolases in obligate heterozygotes. *Humangenetik* 20:119–123, 1973
22. VAN ELSEN AF, LEROY JG, VANNEUVILLE FJ, VERCRUYSSEN AL: Isoenzymes of serum *N*-acetyl-β-D-glucosaminidase in the I-cell disease heterozygote. *Humangenetik* 31:75–81, 1976
23. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275, 1951
24. GLASER JH, SLY WS: β-Glucuronidase deficiency mucopolysaccharidosis: methods for enzymatic diagnosis. *J Lab Clin Med* 82:969–977, 1973
25. BAUM H, DODGSON KS, SPENCER B: The assay of arylsulfatase A and B in human urine. *Clin Chim Acta* 4:453–455, 1969
26. MILLER AL, FREEZE HH, KRESS BD: I-cell disease, in *Lysosomes and Lysosomal Storage Diseases*, edited by CALLAHAN JW, LOWDEN JA, New York, Raven Press, 1981, pp 271–287
27. GLASER JH, MCALISTER WH, SLY WS: Genetic heterogeneity in multiple lyososomal hydrolase deficiency: mucolipidosis II and mucolipidosis III. *J Pediatr* 85:192–198, 1974
28. THOMAS GH, MILLER CS, TOOMEY KE, ET AL.: Two clonal cell populations (mosaicism) in a 46,XY male with mucolipidosis II (I-cell disease)—an autosomal recessive disorder. *Am J Hum Genet* 34:611–622, 1982

29. KABACK MM, ZEIGER RS, REYNOLDS LW, SONNEBORN M: Approach to the control and prevention of Tay-Sachs disease, in *Progress in Medical Genetics*, vol 10, edited by STEINBERG AG, BEAM A, New York, Grune and Stratton, 1974, pp 103–134
30. JOLLY RD, DESNICK RJ: Inborn errors of lysosomal catabolism—principles of heterozygote detection. *Am J Med Genet* 4:293–307, 1979

SYMPOSIUM HONORING DR. C. C. LI. The Graduate School of Public Health, University of Pittsburgh, is sponsoring a symposium honoring the distinguished geneticist Dr. C. C. Li, to be held in Pittsburgh on Monday, October 4, and Tuesday, October 5, 1982, immediately following the annual meeting of the American Society of Human Genetics. A list of participants include the following: Dr. L. L. Cavalli-Sforza, Dr. C. S. Chung, Dr. James F. Crow, Dr. Robert C. Elston, Dr. Cathy Falk, Dr. Samuel Karlin, Dr. Jean MacCluer, Dr. Newton E. Morton, Dr. James V. Neel, Dr. Masatoshi Nei, Dr. C. R. Rao, Dr. D. C. Rao, and Dr. Sewall Wright. For registration forms or further information, please write to either: Dr. Aravinda Chakravarti, University of Pittsburgh, Graduate School of Public Health, Department of Biostatistics, Pittsburgh, PA 15261; or Dr. Kenneth L. Garver, Magee-Women's Hospital, Department of Reproductive Genetics, Pittsburgh, PA 15213.