Studies on the Defect Underlying the Lysosomal Storage of Sialic Acid in Salla Disease

Lysosomal Accumulation of Sialic Acid Formed From *N*-Acetyl-Mannosamine or Derived from Low Density Lipoprotein in Cultured Mutant Fibroblasts

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Abstract

Salla disease is a lysosomal storage disorder characterized by mental retardation and disturbed sialic acid metabolism. To study endogenous synthesis and breakdown of sialic acid, fibroblasts were incubated for 5 d in the presence and then in the absence of *N*-[^{3}H]acetylmannosamine. Labeling of free sialic acid was 5–10 times higher in mutant than in normal cells. Radioactivity decreased in 4 d by 75% in normal but only by 30% in mutant fibroblasts. The labeling pattern was not normalized upon coculture of mutant and normal cells.

To study the metabolism of extracellular sialic acid, low-density lipoprotein (LDL) was labeled in the sialic acid moiety (periodate-NaB^{3}H$_4$) or in the protein moiety (^{125}I). Binding, internalization, lysosomal degradation, and exit of products of protein catabolism were similar in normal and mutant fibroblasts. Upon incubation with LDL labeled in the sialic acid moiety, mutant cells accumulated 2–3 times more free sialic acid radioactivity than normal fibroblasts, mostly in the lysosomal fraction. After a 24-h chase incubation, radioactivity in free sialic acid decreased by 70–80% in normal but only by 10–30% in mutant cells. In mutant fibroblasts, 40% of the radioactivity remained in lysosomes, whereas no labeled free sialic acid was detected in lysosomes from normal fibroblasts.

We conclude that in Salla disease, fibroblast endogenous synthesis of sialic acid and lysosomal cleavage of exogenous glycoconjugates is normal, but free sialic acid cannot leave the lysosome. These findings suggest that the basic defect in Salla disease is deficient transport of free sialic acid through the lysosomal membrane.

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Introduction

Salla disease is a recessively inherited lysosomal storage disease. The main clinical features are early onset psychomotor retardation and ataxia, slow progression of the disease, and almost normal life expectancy (1, 2). More than 60 patients have been identified in Finland, and recently, two cases were identified in Sweden (Ylitalo, V., J. Rapola, I. E. Månsson, L. Svennerholm, G. Sanner, B. Tonnby, and B. Hagberg, manuscript submitted for publication).

In Salla patients, the concentration of free sialic acid in tissues is 15–30-fold increased (3) and large amounts of this compound are excreted in the urine (4). Electron microscopic and histochemical studies have indicated that sialic acid accumulates in the lysosome (1, 2, 5). Biochemical studies on liver tissue and cultured fibroblasts have not revealed defects in the activities of the main cellular enzymes involved in sialic acid metabolism (3). Also, the concentrations of glycopeptides, gangliosides, and neutral lipids in liver tissue from patients with Salla disease are normal (3). These findings have led to the hypothesis (3) that the defect in Salla disease is an inability to release into the cytoplasm the sialic acid, which is normally liberated in lysosomes from glycoproteins and glycolipids by neuraminidase.

A disorder resembling Salla disease, generalized *N*-acetylneuraminic acid storage disease, with analogous abnormalities in lysosomal sialic acid storage and urinary excretion but a more severe clinical course, has also been described (6–10). In this condition cellular glycoconjugate composition is normal, and defects of certain key enzymes of sialic acid metabolism have been ruled out. An abnormal pattern of incorporation of *N*-[^{3}H]acetylmannosamine into cellular free sialic acid has been described (9).

We have studied the accumulation and disappearance of cellular and lysosomal sialic acid, either formed in the cell endogenously or transported into the lysosome from outside the cell. To rule out a more generalized defect in lysosomal protein and lipid degradation, we chose to study the binding, internalization, and lysosomal breakdown of the low density lipoprotein (LDL) (11). Because of its relatively high sialic content (12), it

was considered to be a suitable vehicle for sialic acid transport into the lysosome.

In this report we describe increased intracellular accumulation of sialic acid formed endogenously from a labeled precursor, *N*-acetylmannosamine. We also demonstrate that, in mutant cells, LDL-derived free sialic acid accumulates in the lysosomal fraction, whereas the protein moiety of LDL is catabolized normally.

Methods

Subjects. The three patients studied fulfilled the diagnostic criteria for Salla disease: (*a*) mental retardation; (*b*) lysosomal storage demonstrated by electron microscopy in fresh skin biopsy specimens; (*c*) increased excretion of free sialic acid in the urine. The clinical findings have been reported in detail (2, 13). Urinary excretion of free sialic acid in the patients was increased about 10-fold, ranging from 50 to 200 mg/24 h (controls; 5–15 mg/24 h). In confluent fibroblast cultures from the patients, the amount of free sialic acid was 7–10 μg/mg protein, representing about 70% of the total amount of sialic acid. In control fibroblasts the amount of free sialic acid was <1 μg/mg protein.

Cell culture. Skin fibroblasts from the patients and three normal controls were cultured under standard conditions using Ham's F-10 medium supplemented with 10% fetal calf serum, penicillin (50 U/ml), and glutamine (2 mM). The control and mutant fibroblasts were matched for passage level and age of the donor. Human LDL receptor-negative familial hypercholesterolemia fibroblasts (GM 1915 B) were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ).

Incorporation of N-[^{3}H]acetylmannosamine into sialic acid. To study endogenous synthesis of sialic acid, confluent fibroblasts were incubated for 1–5 d in media containing 10 μCi/ml of *N*-[^{3}H]acetyl-D-mannosamine (22.8 Ci/mmol, New England Nuclear, Boston, MA). To follow the disappearance of cellular radioactivity, cultures labeled for 5 d were washed three times with fresh media and three times with phosphate-buffered saline (PBS). Thereafter, the culture was continued for 2–4 d in media without the isotope. The cells were harvested by trypsinization and processed for sialic acid determination.

Similar labeling experiments were performed with L-[^{14}C]leucine (348 mCi/mmol; The Radiochemical Center, Amersham Ltd., Amersham, United Kingdom), using 50 nCi/ml medium. After incubation with [^{14}C]leucine, the cells were harvested and centrifuged at 600 *g* for 10 min. The pellets were sonicated in water, their protein-containing material precipitated with 10% trichloroacetic acid, suspended in 0.9 M NaOH, and the radioactivity determined.

Co-culture of normal and mutant fibroblasts. Equal amounts (5×10^5 cells) of fibroblasts from a female control (age, 30 yr) and a male Salla patient (age, 33 yr) were co-cultured until confluency, and thereafter, used for *N*-[^{3}H]acetyl-mannosamine incorporation studies as described above. Equal growth of both cell lines was confirmed using Y-body staining.

Isolation and radioactive labeling of LDL. Blood was obtained in 0.1% EDTA after a 12 to 14-h fast and centrifuged. The LDL fraction ($1.019 < d < 1.05$ g/ml) and the lipoprotein-deficient serum ($d > 1.21$ g/ml) were prepared by sequential ultracentrifugation (14).

The sialic acid content of LDL purified from Salla and control serum was in the same range, 8–12 μg/mg protein (12).

The sialic acid portion of LDL from normal serum was labeled as described (15, 16). In short, 10 mg of LDL in 0.5 ml of PBS, pH 7.4, was incubated with 10 μl of 0.1 M Na-periodate (Merck, Darmstadt, Federal Republic of Germany) for 10 min at 0°C. The periodate was removed by gel filtration on a 1.5 × 20-cm column of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with PBS, pH 8.0. The LDL was then incubated with 20 mCi of tritiated sodium borohydride (15 Ci/mmol; The Radiochemical Center, Amersham, Ltd.) for 60 min at 4°C. This treatment converts the sialic acid moiety into its closely related ^{3}H-labeled analogue, 5-acetamido-3,5-dideoxy-L-arabino-2-heptulosonic acid (15, 16), referred to as [^{3}H]sialic acid. The ^{3}H-labeled LDL (sp act 60–120 cpm/ng of protein) was separated from NaB^3H_4 by gel filtration. After overnight dialysis against PBS, the lipoprotein was sterilized by passing it through a 0.22-μm filter (Millipore Continental Water Systems, Bedford, MA) and stored at 4°C. The specific activity of the labeled LDL was 60–120 cpm/ng of protein.

To verify the labeling of the sialic acid portion, the radioactive LDL was subjected to hydrolysis in 0.1 M H_2SO_4 at 80°C for 60 min, which liberates sialic acid (17). After hydrolysis, gel filtration on a Sephadex G-50 column (2.5 × 50 cm) (Pharmacia Fine Chemicals) in PBS, pH 7.4, revealed that all of the radioactivity had been released from LDL and appeared as a single peak at a similar elution volume as free sialic acid. This radioactive material was further characterized by thin layer chromatography and ion exchange chromatography (17), as outlined below. About 90% of the radioactivity released from the LDL was attributable to modified sialic acid. Neuraminidase (Vibrio comma, Koch-Light Laboratories Ltd., Colnbrook, United Kingdom) treatment of radioactive LDL at 37°C for 2 h liberated more than 80% of the radioactivity.

LDL was radiolabeled with ^{125}I using the iodine monochloride method (18), as modified for lipoproteins (19). The specific activity in different preparations varied between 100 and 150 cpm/ng protein.

Binding, internalization, and degradation of labeled LDL. Fibroblasts were seeded at a concentration of 1×10^5 cells/dish into 35 × 15-mm dishes containing 2 ml of standard growth medium. On day 3, when the cells were in early logarithmic growth, monolayers were washed with PBS and 2 ml of fresh medium containing 10% (vol/vol) lipoprotein-deficient human serum (hLPDS)[1] were added. After a 48-h incubation with hLPDS (day 5), the medium was replaced by fresh medium containing either ^{125}I- or ^{3}H-labeled LDL (10 μg protein/ml of medium containing 10% hLPDS). After incubation with ^{125}I-labeled LDL, the medium was treated with hydrogen peroxide, extracted with chloroform to remove free iodine (20), and counted to determine the amount of ^{125}I-labeled, acid-soluble material formed by the cells and released to the medium (21). The cell monolayers were treated with heparin and the amount of ^{125}I-labeled LDL that had been bound to LDL-receptors and released by heparin was counted (22). After the incubation with heparin, the monolayer of cells was dissolved in 0.2 N NaOH, and an aliquot was counted to determine the amount of LDL internalized.

The incubation with ^{3}H-labeled LDL started on day 5 and continued for 2 to 48 h. In chase experiments, cells were first incubated for 24 h with ^{3}H-labeled LDL, washed, and further incubated in fresh medium containing unlabeled LDL for up to 24 h. After removal of the culture medium, cells were washed three times with 2 ml PBS containing 1% albumin and three times with 2 ml PBS, and harvested by trypsinization.

Determination of sialic acid. To determine cellular radioactive sialic acid, washed cell pellets were suspended in water and disrupted by brief sonication. The sonicate was centrifuged at 100,000 *g* for 60 min, and the supernatant was sequentially chromatographed on columns containing Dowex 50-×8 (200–400 mesh) in H^+ form and Dowex 2-×8 (200–400 mesh) (Fluka AG, Buchs, Switzerland) in acetate form (0.4 ml of each resin) (17). Sialic acid-containing material was eluted from the Dowex 2-×8 columns with 0.2 M ammonium acetate (13) and its radioactivity determined. Aliquots of the eluate were lyophilized, applied with authentic *N*-acetylneuraminic acid (Sigma Chemical Co., St. Louis, MO) on thin-layer chromatography plates (Merck), and developed in either *n*-butanol/acetic acid/water (2:1:1, vol/vol/vol) or *n*-propanol/water (7:3, vol/vol). Sialic acid was visualized with a resorcinol spray reagent (23) and scraped for counting of radioactivity. Sialic acid was measured chemically by the thiobarbituric acid method (24), using *N*-acetylneuraminic acid as standard.

Preparation of lysosome-enriched subcellular fractions. After a 24-h incubation with ^{3}H-labeled LDL, fibroblasts were washed, harvested with a rubber policeman, and suspended in 1 ml of 0.25 M sucrose/1 mM

1. *Abbreviations used in this paper:* hLPDS, human lipoprotein-deficient serum; SEAT, 0.25 M sucrose/1 mM EDTA per 10 mM acetic acid per 10 mM triethanolamine buffer, pH 7.4.

EDTA per 10 mM acetic acid per 10 mM triethanolamine buffer, pH 7.4 (SEAT) (25). Cells were then passed 50 times through the tip of a 1-ml Eppendorf pipette, and lysis was monitored by microscopy after staining with May-Gruenwald-Giemsa. The lysate was centrifuged at 1,500 *g* for 10 min. The supernatant was mixed with Percoll and SEAT buffer (26) to a final concentration of 35% Percoll (vol/vol) (total vol, 25 ml) and centrifuged in a Sorvall SS 34 fixed angle rotor for 2 h at 35,000 *g* at +4°C. 1-ml fractions of the gradient were collected from the bottom of the tube. Total and sialic acid radioactivity were determined from the fractions and from aliquots taken at each step during preparation. The activity of the lysosomal hydrolase *N*-acetyl-β-hexosaminidase was determined using the 4-methylumbelliferyl derivative as substrate (1).

Protein was determined as described (27).

Results

Metabolism of endogenously synthesized sialic acid. Confluent fibroblasts were incubated with *N*-[^{3}H]acetylmannosamine, a precursor of sialic acid. After 5 d of incubation, the total amount of radioactivity incorporated into mutant cells was only slightly higher than that into control fibroblasts (data not shown).

The amount of labeled free sialic acid did not increase after 2 d of incubation in control cells. In contrast, the labeling of free sialic acid in mutant fibroblasts continued to increase throughout the incubation and at 5 d was 5–10 times higher than in control cells (Fig. 1). During a 4-d chase incubation without labeled precursor, the amount of labeled sialic acid in control cells decreased to ~25%, but in Salla fibroblasts, it decreased to only ~70% of that observed on day 5 (Fig. 1). Neither mutant nor control fibroblasts secreted detectable amounts of labeled free sialic acid into the culture media.

To rule out a general difference in the synthetic capabilities of the cell lines, incorporation of [^{14}C]leucine into trichloroacetic acid precipitable material was studied. No differences in labeling could be observed between mutant and control fibroblasts during 1–5 d of culture (data not shown).

Co-culture of mutant and control fibroblasts. To assess

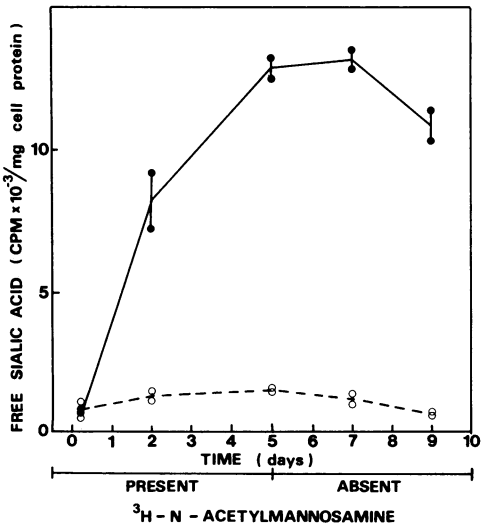

Figure 1. Accumulation of free sialic acid radioactivity in fibroblasts from Salla patients and controls. Confluent mutant (●) and control (○) fibroblasts were incubated in standard growth media with or without 10 μCi/ml *N*-[^{3}H]acetyl-D-mannosamine for the time period indicated. The cells were washed, harvested by trypsinization, and processed for determination of sialic acid radioactivity as described in Methods.

Table I. Co-culture of Salla and Control Fibroblasts

	cpm/mg protein	
Salla	8,916	9,067
Co-culture	4,461	4,102
Control	1,274	1,233

Equal amounts of either cell line were seeded separately or in the same culture flask, and cultivated until confluency. The cells were incubated for 5 d in the presence of (^{3}H-ManNAc), and the sialic acid radioactivity determined from cell pellets sonicates after purification on Dowex 2 × 8 columns.

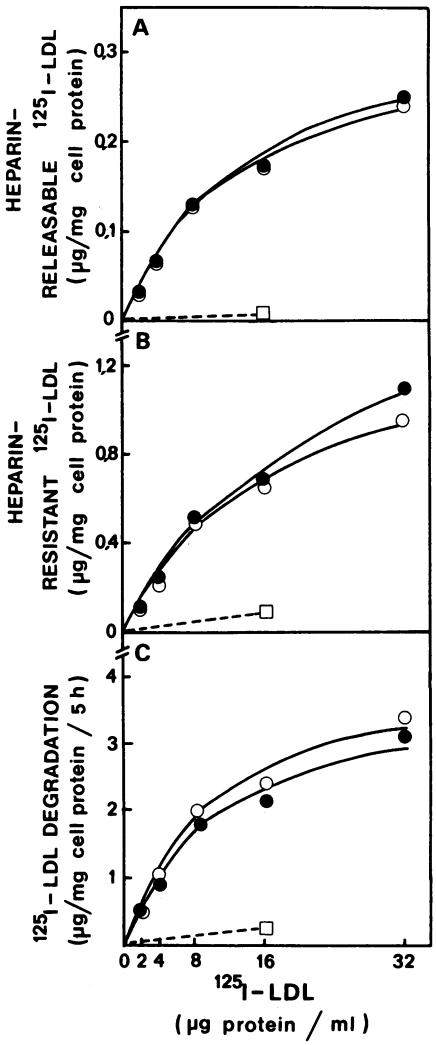

Figure 2. Cell surface binding (*A*), internalization (*B*), and degradation (*C*) of ^{125}I-labeled LDL in fibroblasts from patients and controls. On day 5 of cell growth, each monolayer received 2 ml of medium containing the indicated concentration of ^{125}I-labeled LDL (134 cpm/ng). After incubation for 5 h at 37°C, the medium was removed, and its content of ^{125}I-labeled trichloroacetic acid soluble material (*C*) was measured. The cell monolayers were then washed and the amounts of heparin-releasable (surface-bound; *A*) and heparin-resistant (internalized; *B*) ^{125}I-labeled LDL were determined as described in Methods. ●, Salla disease fibroblasts; ○, control cells; □, cells from a patient with homozygous familial hypercholesterolemia.

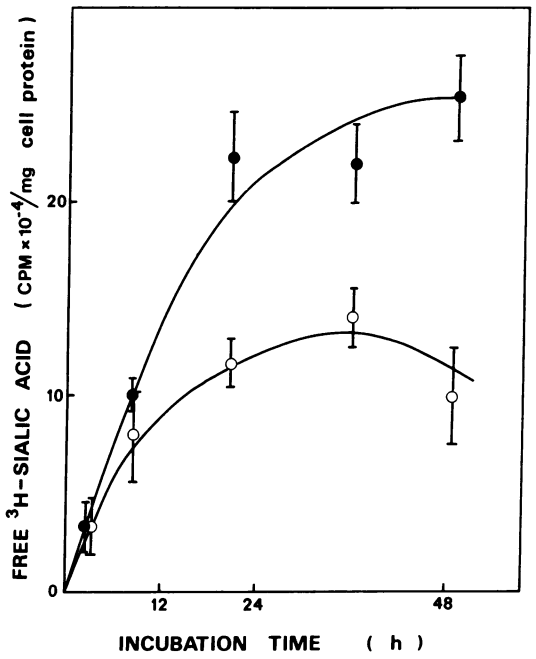

Figure 3. Accumulation of free [^{3}H]sialic acid from sialic acid-labeled LDL into fibroblasts from Salla patients and controls. Fibroblasts were incubated for 48 h in 10% hLPDS, after which the medium was replaced with medium containing 10% hLPDS and 10 μg/ml of ^{3}H-labeled LDL, and incubated for the time periods indicated. After washing, cells were harvested by trypsinization and free [^{3}H]sialic acid determined as described in Methods. ●, mutant cells; ○, control cells.

whether endocytosis of a "correcting factor" provided by normal fibroblasts (28) could prevent sialic acid accumulation in mutant cells, confluent fibroblasts from a male Salla patient and a female control were incubated together for 5 d in the presence of *N*-[^{3}H]acetylmannosamine. After co-culture, approximately 45% of the cells stained for Y-bodies, indicating a similar growth rate for mutant and control cells. The incorporation of radioactivity into free sialic acid (means of two experiments) was 8,980 cpm/mg cell protein in mutant cell cultures, whereas control fibroblasts incorporated 1,250 cpm/mg protein. In mixed fibroblast cultures the amount of labeled free sialic acid was 4,280 cpm/mg protein (Table I). This value, which is intermediate between mutant and control cultures, indicates that no correction of the defect in sialic acid metabolism had resulted from the co-culture.

Metabolism of exogenously provided sialic acid. In order to study whether LDL could be used as a vehicle to deliver sialic acid into lysosomes, we compared the binding and uptake of ^{125}I-labeled LDL in fibroblasts from controls to those from Salla disease or homozygous familial hypercholesterolemia (Fig. 2 *A* and *B*). Both these processes were similar in Salla and control cells. The lysosomal degradation of LDL protein was also similar in these two kinds of fibroblasts, as deduced from the release of trichloroacetic acid-soluble radioactivity from the cells (Fig. 2 *C*), indicating that the LDL receptor pathway in Salla fibroblasts is normal.

In experiments using LDL labeled in the sialic acid portion, the cells were incubated for 2 d in hLPDS and then in a medium containing ^{3}H-labeled LDL for up to 48 h. The binding and internalization of sialic acid-labeled LDL were identical in mutant and control fibroblasts (data not shown). The amount of free [^{3}H]sialic acid increased in a similar fashion in both cell lines during the first hours of incubation, but thereafter more free [^{3}H]sialic acid accumulated in the mutant cells (Fig. 3). This difference increased on further incubation, and at 48 h, the amount of [^{3}H]sialic acid in mutant cells was 2–3 times higher than that observed in control cells, indicating altered intracellular metabolism of sialic acid.

To study the disappearance of free [^{3}H]sialic acid from cells labeled with ^{3}H-labeled LDL, the cultures were washed after a 24-h incubation and further cultured in media containing unlabeled LDL (Fig. 4). In control cells the amount of free [^{3}H]sialic acid decreased rapidly during the chase; at 6 h, ~50% remained and at 24 h, ~20–30% remained. In mutant cells the amount of free [^{3}H]sialic acid further increased during the first 6–8 h of chase incubation, and thereafter, started to decline. After a 24-h chase, ~70–90% of the sialic acid still remained.

Intracellular localization of labeled sialic acid. To determine the intracellular localization of free sialic acid, cells were incubated with ^{3}H-labeled LDL for 24 h, homogenized, and subjected to Percoll density gradient centrifugation. *N*-acetyl-β-hexosaminidase was used as a lysosomal marker enzyme. In the mutant cell lines, the lysosome-enriched fraction appeared at a density of 1.05–1.07 g/ml, whereas the lysosomes of control fibroblasts had a somewhat higher density, 1.06–1.08 g/ml. In the mutant cells, the major part of the total radioactivity in the fractions occurred together with the peak of enzyme activity (Fig. 5). When the radioactivity of free sialic acid was determined, this also

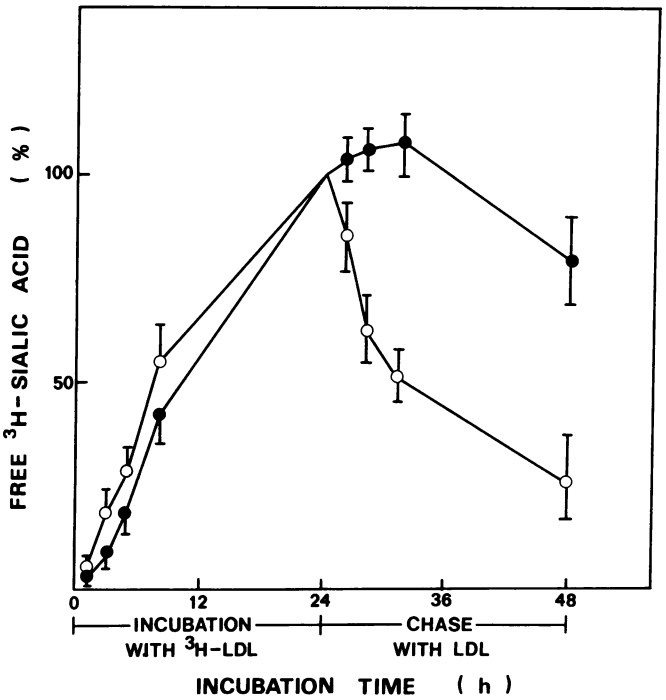

Figure 4. Free [^{3}H]sialic acid radioactivity in fibroblasts from Salla patients and controls after incubation with ^{3}H-labeled LDL followed by incubation with unlabeled LDL. Cells were first incubated with LDL labeled in the sialic acid moiety for 24 h as described in the legend for Fig. 3, and then in media containing unlabeled LDL (10 μg protein/ml medium) for an additional 24 h. The cellular free [^{3}H]sialic acid was determined at the time intervals indicated. The values are expressed as percent of the amount measured at 24 h (absolute values, 190,000–280,000 and 120,000–220,000 cpm/mg protein in mutant and control cells, respectively). ●, mutant fibroblasts; ○, control cells. The values are means and ranges of five experiments.

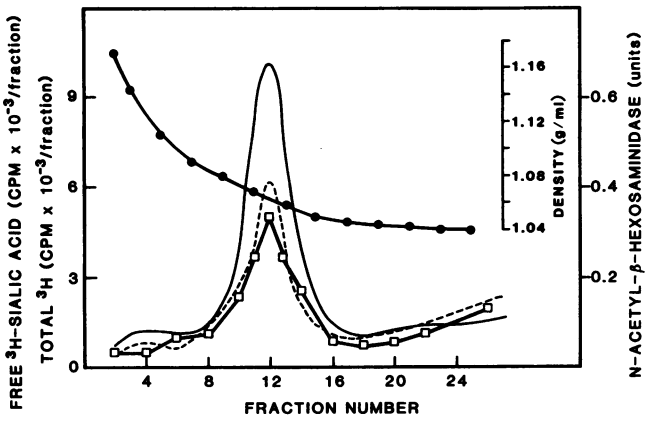

Figure 5. Percoll density gradient fractionation of mutant fibroblast homogenates after a 24-h incubation with ^{3}H-labeled LDL. Preconfluent fibroblasts were labeled as described in the legend for Fig. 3, harvested by scraping, homogenized in SEAT buffer, and centrifuged at 1,500 *g* for 10 min. The supernatant was centrifuged at 35,000 *g* for 2 h at 4°C in 35% (vol/vol) Percoll/SEAT (total vol, 25 ml). The fractions (1 ml) were assayed for *N*-acetyl-β-hexosaminidase (- - -), total radioactivity (——), and free [^{3}H]sialic acid (—□—). The density of the fractions was determined by refractometry (-●-).

peaked with *N*-acetyl-β-hexosaminidase, indicating that in mutant cells most of the free sialic acid is located in the lysosome (Fig. 5). After a 24-h chase incubation in medium containing nonradioactive LDL, ~40% of the initial free [^{3}H]sialic acid could still be detected in the lysosome-enriched fractions from mutant cells, but none in those from control cells (data not shown).

Discussion

Salla disease is a lysosomal storage disorder (1, 2) in which free sialic acid accumulates in tissues and cells (3, 5). We have further characterized this storage phenomenon as well as the lysosomal metabolism of endogenously synthesized and extracellularly supplied sialic acid in cultured fibroblasts (Fig. 6).

Salla disease fibroblasts utilize extracellular *N*-acetylmannosamine for the synthesis of sialic acid at an initial rate similar to that in normal cells. This agrees with the previous finding of normal amounts and degrees of sialylation of tissue gangliosides and glycopeptides (3). Also, sialylated membrane glycoproteins of Salla disease fibroblasts are indistinguishable from normal controls (unpublished observations), suggesting that increased synthesis does not account for sialic acid storage.

Upon prolonged incubation with *N*-[^{3}H]acetylmannosamine, more free sialic acid accumulates in mutant cells than in normal fibroblasts. Since the disappearance of labeled sialic acid is slower in mutant than in normal cells and since no labeled sialic acid appears in the medium from either cell type, delayed intracellular processing of free sialic acid seems likely.

Cultured fibroblasts secrete lysosomal enzymes into the medium and take them up through receptor-mediated endocytosis (29). Several lysosomal disorders can be corrected by co-culture with normal fibroblasts (28). No correction of free [^{3}H]sialic acid storage after incubation with *N*-[^{3}H]acetylmannosamine was seen in Salla disease fibroblasts co-cultured with normal cells, suggesting that the defect is not related to a secreted and endocytosed product.

The synthesis of sialic acid from *N*-acetylmannosamine involves several reactions (Fig. 6), and only ~5% of the precursor

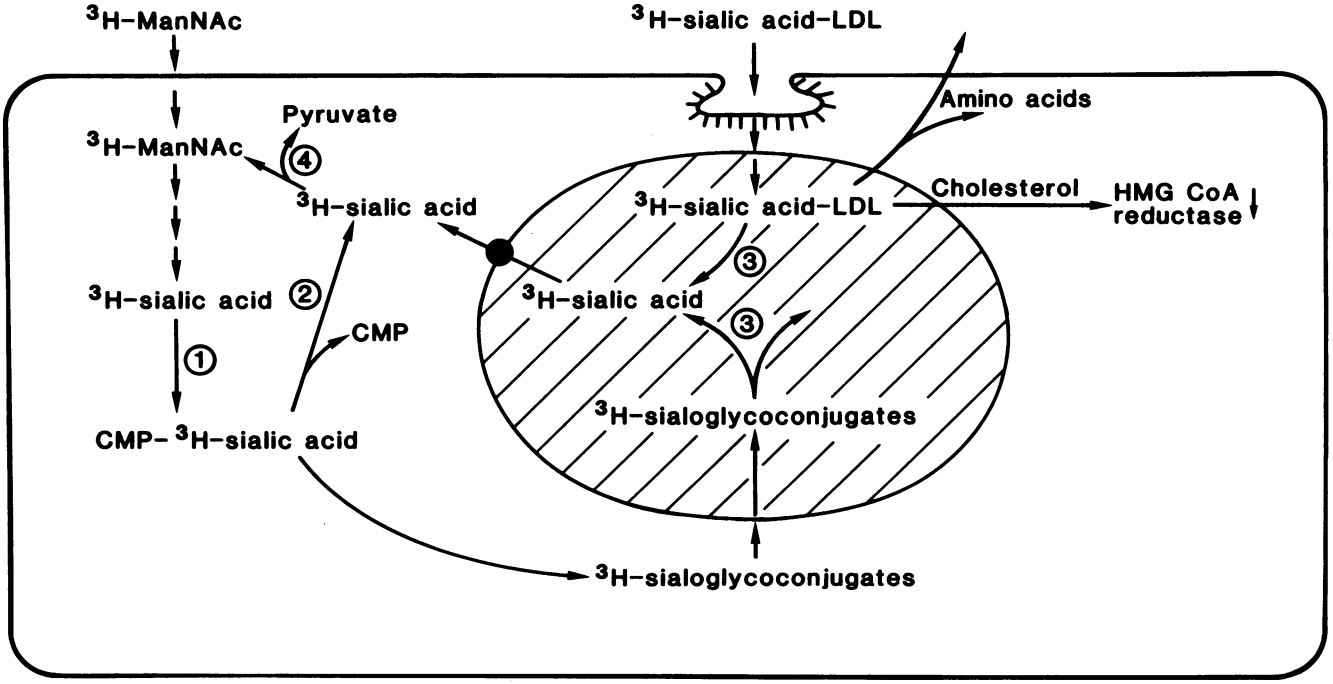

Figure 6. Outline of metabolic pathways of *N*-[^{3}H]acetylmannosamine (^{3}H-ManNAc) and of low density lipoprotein labeled in the sialic acid moiety ([^{3}H]sialic acid-labeled LDL) in a fibroblast and its lysosome (shaded). The numbered enzymes have been reported to have normal activities in Salla disease and/or generalized *N*-acetylneuraminic acid storage disease (3, 9). *1*, CTP-*N*-acylneuraminate cytidylyltransferase; *2*, CMP-*N*-acylneuraminate *N*-acylneuraminohydrolase; *3*, Neuraminidase; *4*, N-acetylneuraminate pyruvate lyase. The postulated metabolic defect in Salla disease, i.e., defective release of free sialic acid from the lysosome, is indicated with the black circle. HMG CoA reductase; hydroxymethylglutaryl coenzyme A reductase.

was converted into free sialic acid in our studies. These results did not reveal whether the intracellular free sialic acid pool was labeled directly or after initial incorporation into glycoconjugates, nor whether the accumulation was predominantly lysosomal.

Using LDL labeled in the sialic acid portion to specifically load lysosomes, we demonstrated that free sialic acid accumulates within the lysosomes and is released more slowly in mutant fibroblasts compared with normals.

These findings suggest that lysosomal neuraminidase releases sialic acid from the glycoconjugates of LDL, and that free sialic acid is unable to leave the lysosomal compartment at a normal rate. As a result, its catabolism is prevented in that the requisite enzyme, *N*-acetylneuraminate pyruvate lyase, is located in the cytoplasm (3, 30).

These observations support the hypothesis (3) that the basic defect in Salla disease involves the transport of free sialic acid out of the lysosome. A generalized abnormality of the lysosomal membrane appears unlikely because the products of LDL protein catabolism were released in a normal way. Neutral monosaccharides and amino acids are considered to cross the lysosomal membrane by passive diffusion (31–33), but facilitated diffusion of certain monosaccharides has also been reported (34). The molecular mechanisms for the release of charged compounds are less well known. Lysosomal transport of the basic amino acid cystine is carrier-mediated and is defective in cystinosis (35, 36). Sialic acid, a negatively charged compound (pK 2.6), may also require a specific transport mechanism.

Three groups of disorders involving sialic acid metabolism have been described. In sialidosis, a defect in acid neuraminidase, with or without a concomitant β-galactosidase deficiency, leads to lysosomal accumulation of sialylated glycoconjugates (37, 38). In sialuria (39), which is characterized by massive excretion of free sialic acid, defective feedback inhibition of one of the enzymes involved in sialic acid synthesis has been implicated (40). In this unique patient, no lysosomal storage occurs.

The third type of abnormality includes Salla disease and the clinically more severe generalized sialic acid storage disease (6–10). This latter disorder may represent a more extensive biochemical defect similar to that of Salla disease.

Fibroblasts have an efficient mechanism for the utilization of LDL, which involves lysosomal uptake and processing. This investigation has demonstrated that LDL can be used to specifically load fibroblast lysosomes with sialic acid for the subsequent measurement of its egress. The technique may be useful in the prenatal detection of Salla disease, and may also be applied to the study of other lysosomal storage disorders.

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