Type C Niemann-Pick Disease

LYSOSOMAL ACCUMULATION AND DEFECTIVE INTRACELLULAR MOBILIZATION OF LOW DENSITY LIPOPROTEIN CHOLESTEROL*

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The intracellular accumulation of unesterified cholesterol was examined during 24 h of low density lipoprotein (LDL) uptake in normal and Niemann-Pick C fibroblasts by fluorescence microscopy with filipin staining and immunocytochemistry. Perinuclear fluorescence derived from filipin-sterol complexes was observed in both normal and mutant cells by 2 h. This perinuclear cholesterol staining reached its peak in normal cells at 6 h. Subsequent development of fluorescence during the remaining 18 h of LDL incubation was primarily limited to the plasma membrane region of normal cells. In contrast, mutant cells developed a much more intense perinuclear fluorescence throughout the entire 24 h of LDL uptake with little enhancement of cholesterol fluorescence staining in the plasma membranes. Direct mass measurements confirmed that internalized LDL cholesterol more readily replenishes the plasma membrane cholesterol of normal than of mutant fibroblasts. Perinuclear filipin-cholesterol fluorescence of both normal and mutant cells was co-localized with lysosomes by indirect immunocytochemical staining of lysosomal membrane protein.

Abnormal sequestration of LDL cholesterol in mutant cells within a metabolically latent pool is supported by the finding that in vitro esterification of cellular cholesterol could be stimulated in mutant but not in normal cell homogenates by extensive disruption of the intracellular membranous structures of cells previously cultured with LDL.

Deficient translocation of exogenously derived cholesterol from lysosomes to other intracellular membrane sites may be responsible for the delayed homeostatic responses associated with LDL uptake by mutant Niemann-Pick Type C fibroblasts.

Type C Niemann-Pick disease is a human autosomal-recessive lipid storage disorder (1). Certain clinical, morphological, and biochemical similarities with type A and B disorders prompted the classification of this disorder as Type C Niemann-Pick disease (2). Although subsequent studies showed both the type A and B disorders to be primary sphingomyelinase mutations (3–5), no consistent evidence of a similar primary lesion in sphingomyelin catabolism has been reported for Type C Niemann-Pick disease (6). To the contrary, recent investigations have suggested that this disorder may, in fact, represent a primary lesion that disturbs critical balances in cholesterol metabolism (7–11).

Cellular cholesterol homeostasis involves a series of integrated responses that enable cells to maintain cholesterol levels within a critical range needed for optimal growth and development under environmental conditions that include both cholesterol excess and deprivation (12). Receptor-mediated uptake and hydrolytic lysosomal processing of LDL in cultured fibroblasts derived from Niemann-Pick C patients are associated with cellular homeostatic responses that are uniformely delayed (11). Lipoprotein uptake by the mutant cells leads to an excessive intracellular accumulation and storage of cholesterol primarily as unesterified sterol (8). The inability of internalized cholesterol to initiate timely regulatory responses in these mutant cells could have resulted from a primary lesion either in the initiation of a regulatory message commonly shared by all the affected responses or in the intracellular transport of cholesterol. The present data will document that a sterol transport error plays a major role in the cellular pathology of Niemann-Pick C disease.

EXPERIMENTAL PROCEDURES

Materials—[1,10-3H]Oleic acid (2–10 Ci/mmol) was obtained from Du Pont-New England Nuclear. ATP, CoA, fatty acid-free bovine serum albumin, and human LDL were purchased from Sigma. Pre-coated silica gel 60 plates were obtained from Merck. The enzymes used in the direct fluorometric assay of cholesterol masses, cholesterol oxidase, and xanthinase/peroxidase were purchased from Boehringer Mannheim. Lipoprotein-deficient fetal bovine serum (LPDS) was prepared by Biomedical Technologies, Boston, from KBr serum solutions by ultracentrifugation as described (8). ITS (insulin/transferrin/selenium) was obtained from Collaborative Research, Inc., Bedford, MA.

Cell Cultures—Normal and mutant Type C Niemann-Pick fibroblasts represented established secondary cell lines derived from superficial skin biopsies of normal volunteers and confirmed patients of the Developmental and Metabolic Neurology Branch of the National Institutes of Health. Cell cultures were maintained in Eagle's

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1 The abbreviations used are: LDL, low density lipoprotein; LPDS, bovine lipoprotein-deficient serum.
minimal essential medium supplemented with 10% (v/v) complete fetal bovine serum, 2 mM l-glutamine, 100 units of penicillin, and 100 μg streptomycin/ml in humidified 95% air and 5% CO2 at 37 °C. Cells were harvested by washing monolayers three times with phosphate-buffered saline (PBS) and subsequent treatment with 0.05% trypsin (Sigma) for 3 min at 37 °C. Specific experimental culture manipulations and conditions are described in the appropriate legends.

Fluorescent Cytochemical and Immunocytochemical Staining of Cholesterol and Lysosomes—Cells were seeded and cultured directly on microscope slide chambers (Lab Tek). In experiments designed to measure only unesterified cholesterol, cell monolayers were fixed with 10% phosphate-buffered formalin and subsequently stained with 0.05 mg/ml of filipin (generously supplied by The Upjohn Co.) for 60 min as described previously (8). Fluorescence of stained preparations was photographed with excitation from a 100-watt mercury arc lamp passed through UG-1 filter and emission viewed through a 510-nm filter using a 60× exposure. Concurrent filipin-cholesterol staining and rhodamine-labeled anti-lysosomal antibody fluorescence detection were carried out as follows. Cells in the plastic slide chambers were washed with PBS, fixed in 3% paraformaldehyde for 30 min at room temperature, and washed three times with PBS. All subsequent steps were carried out in a 10% solution of normal fetal calf serum in PBS with 0.05 mg/ml of filipin. The use of filipin in all incubation solutions served to permeabilize the cells to the antibody preparations and to fluorescently label unesterified cholesterol. The primary antibody was rat antibody specifically directed against human lysosomal membrane protein and was a generous gift of Dr. J. W. Chen, Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, MD (13). Cells were incubated with the primary antibody at a 1:4 dilution for 30 min. The cells were washed free of unbound primary antibody and incubated with affinity-purified goat anti-rat IgG conjugated to rhodamine (Jackson Labs, Avondale, PA) at a dilution of 1:40 or 30 min. Cells were washed, mounted in para-phenylenediamineglycerol, and viewed with a Leitz fluorescence microscope using an excitation filter (band pass 350-410) for filipin and (band pass 530-560) for rhodamine. Control for the immunocytochemical study was the replacement of the specific primary monoclonal antibody to human lysosomal membrane protein with a nonspecific monoclonal antibody to mouse lysosomal membrane protein and subsequent treatment of the cells with rhodamine-conjugated antibody. No rhodamine fluorescence was noted with the human cells. Controls for discrete visualization of fluorescent signal were: (1) cells exposed to filipin alone and viewed to the rhodamine (band pass 530-560) showed no signal; (2) cells exposed to specific primary antibodies and second rhodamine-conjugated IgG using saponin as the permeabilizing agent and viewed at the filipin (band pass 350-410) showed no signal. Determination of Unesterified Cholesterol in Plasma Membranes—Advantage was taken of the observation of Lange (14, 15) that cholesterol oxidase efficiently and selectively oxidizes only plasma membrane cholesterol when intact cell suspensions are first treated with glutaraldehyde. Freshly harvested cell pellets (5 mg of protein) were suspended in 1 ml of PBS and aliquoted into separate 200-μl samples. Cells were pelleted and resuspended in 0.20 ml of PBS ± 1% glutaraldehyde. Following gentle mixing and incubation in an ice bath for 10–15 min, cells were pelleted by low speed centrifugation and washed three times with 1 ml of 310 mM sucrose and 0.5 mM phosphate buffer, pH 7.5, at 10 °C. The individual cell pellets were homogenized (250 mM sucrose and 10 mM Tris-HCl, pH 7.4) by an extended, prior period of lipo-oxidase treatment of the membrane protein and was a generous gift of Dr. J. W. Chen, Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, MD (13). Cells were incubated with the primary antibody at a 1:4 dilution for 30 min. The cells were washed free of unbound primary antibody and incubated with affinity-purified goat anti-rat IgG conjugated to rhodamine (Jackson Labs, Avondale, PA) at a dilution of 1:40 or 30 min. Cells were washed, mounted in para-phenylenediamineglycerol, and viewed with a Leitz fluorescence microscope using an excitation filter (band pass 350-410) for filipin and (band pass 530-560) for rhodamine. Control for the immunocytochemical study was the replacement of the specific primary monoclonal antibody to human lysosomal membrane protein with a nonspecific monoclonal antibody to mouse lysosomal membrane protein and subsequent treatment of the cells with rhodamine-conjugated antibody. No rhodamine fluorescence was noted with the human cells. Controls for discrete visualization of fluorescent signal were: (1) cells exposed to filipin alone and viewed to the rhodamine (band pass 530-560) showed no signal; (2) cells exposed to specific primary antibodies and second rhodamine-conjugated IgG using saponin as the permeabilizing agent and viewed at the filipin (band pass 350-410) showed no signal.

In Vitro Esterification of Cellular Cholesterol—Freshly harvested and washed cell pellets (5 mg of protein) were suspended in 1 ml of 250 mM sucrose and 10 mM Tris-HCl, pH 7.4. The cell suspensions were divided into separate aliquots of 0.20 ml. Some of the suspensions were centrifuged and the cell pellets frozen in liquid nitrogen for 1 h. The frozen cell pellets were subsequently taken up in 0.20 ml of 10 mM Tris-HCl, pH 7.4, and frozen and thawed an additional three times in liquid nitrogen and at 37 °C at 5-min intervals. These lysed cells were further disrupted by vigorous homogenization for 1 min at 0 °C in a small glass-fritted homogenizing tube with a motorized tight-fitting glass pestle at 1000 rpm. Other portions of the fresh cell suspensions in the isotonic sucrose buffer were placed in a small N2 cavitation chamber (Kontes) at 40 p.s.i. for 5 min at 4 °C. These partially lysed cell suspensions were further homogenized gently in a sonicoud, then resuspended in a loose Teflon pestle at 100 r.p.m. for 30 s at 0 °C. It has previously been shown that such controlled disruption allows cell-free extracts to essentially retain intact subcellular organelles (18). Aliquots (0.010 ml and 50 μg of protein) of the respective total cell-free extracts were incubated in 0.19 μM cholesterol oxidase, 2 mM dithiothreitol, 5 mM KF, and 10 mM Tris-HCl, pH 7.4, containing 6 mM ATP, 0.6 mM CoA, 15 mM MgCl2, and 0.40 mM [3H]oleate (370 dpm/pmol) in 14% fatty acid-free bovine serum albumin. Incubations were carried out for 2 h at 37 °C and the lipids subsequently extracted with chloroform/methanol (2:1 v/v). The level of [3H]oleate incorporated into cellular cholesterol to form cholesterol[3H]oleate was measured by thin layer chromatography as described previously (8).

RESULTS

Fluorescence Microscopic Studies of the Intracellular Storage of Unesterified Cholesterol—LDL uptake was monitored over a period of 24 h in normal and mutant Niemann-Pick C fibroblasts condition by an extended prior period of lipoprotein deprivation. At indicated intervals, cell cultures were washed, fixed, and stained with filipin to follow the intracellular deposition of unesterified LDL-derived cholesterol. (Fig. 1). Prior to the uptake of LDL only low levels of filipin staining were found in the cultures indicating a minimal cellular content of unesterified cholesterol. Following 2 h of LDL uptake, a distinctive perinuclear filipin staining developed in both cell lines which was somewhat more intense in the Niemann-Pick C cells. After 6 h of LDL uptake, perinuclear fluorescence in the mutant cells had increased to levels which were now significantly higher than those in normal cells. Between 6 and 24 h of LDL uptake, a further significant increase of perinuclear filipin-cholesterol staining was noted in mutant but not in normal cells. The current fluorescent studies expand our earlier observations of excessive unesterified cholesterol storage in mutant cells (9, 10) and clearly now document the temporal and sequential fashion of LDL cholesterol accumulation in NF-C cells.
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Fig. 1.
Rhodamine Immuno-Staining of Lysosomes

Filipin Staining for Unesterified Cholesterol

**Fig. 2. Cytochemical localization of unesterified cholesterol and lysosomes in normal and Niemann-Pick C cells cultured with LDL.** Normal and mutant cells were cultured and prepared for LDL uptake as described in the legend to Fig. 1. Cells were incubated with LDL (60 μg/ml) for 24 h and subsequently washed, fixed, and prepared for cytochemical staining of unesterified cholesterol with filipin and immunocytochemical staining of lysosomes with rhodamine-labeled antibodies as described under "Experimental Procedures." Magnification × 94.

The perinuclear storage depots for the unesterified cholesterol were identified as lysosomes (Fig. 2). Filipin fluorescence in the perinuclear region showed a very high degree of identity with a second separate fluorescent signal (rhodamine antibody complex) targeted specifically to the lysosomes.

Previous observations of mutant cells had indicated that excessive perinuclear accumulation of LDL cholesterol was contrasted with a deficient cholesterol replenishment of plasma membranes when compared to normal cells (10). These preliminary observations could now be confirmed with cell cultures that were extensively deprived of medium cholesterol prior to their uptake of LDL (Fig. 3). This extensive prior cholesterol depletion enhances the differences in intensity of filipin staining in LDL-treated and nontreated fibroblasts. Presumably, this pretreatment minimizes endogenous cellular cholesterol levels prior to lipoprotein loading. LDL uptake by normal cells was shown to be associated not only with notable perinuclear filipin staining but also with a fainter but discernable development of filipin staining at the outer plasma membrane region of the cells (Fig. 3). In comparably treated Niemann-Pick C fibroblasts, a significantly lower level of filipin fluorescence staining was noted in the plasma membrane even though very intense filipin-cholesterol staining formed within the perinuclear region of the mutant cells.

Although these photomicrographs are made at a single plane and show a somewhat diffuse intracellular fluorescence at the outer boundaries of the cholesterol-loaded cells, application of through focus analysis at many planes in the cell allowed one to clearly identify a peripheral plasma membrane fluorescent line. Other ongoing studies with filipin at the electron microscopic level indicate that filipin-cholesterol complexes are present in the plasma membrane of normal LDL-loaded cells. Independent biochemical verification of specific plasma membrane cholesterol enrichment is presented in the next section.

**Relative Mass Measurement of Unesterified Cholesterol in**

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**Plasma Membranes**—In order to directly quantitate relative distributions of unesterified cholesterol in the respective intracellular and plasma membrane domains of cultured fibroblasts, advantage was taken of the selective oxidation by cholesterol oxidase of plasma membrane cholesterol in intact, glutaraldehyde-treated cells (14, 15). In cells grown with 10% lipoprotein-deficient serum for 4 days, comparable levels and distributions of unesterified cholesterol were seen in normal and mutant cells with somewhat higher intracellular levels found in the mutant cells (Table I). Following 24 h of LDL uptake, total cellular unesterified cholesterol rose in mutant cells to levels substantially higher than those found in normal cells. However, in association with this internalization of excessive LDL cholesterol by mutant cells, there was less enrichment of the plasma membranes with cholesterol when compared to normal cells.

Both the relative mass measurements as well as fluorescent filipin staining show plasma membrane cholesterol levels to be substantially lower (16–46% of total) than reported by others for cultured fibroblasts (>90% of total) (14, 15). These former studies were carried out with confluent cells cultured with LPDS for 24 h. The present experiments were specifically designed to study intracellular distribution of cholesterol in extensively sterol-depleted, sparsely populated, and actively growing cultures during the active phase of LDL uptake. It is likely that specific culture conditions play a major role in determining the disposition of cellular cholesterol between intracellular and plasma membrane pools.

**Comparative Esterifications of Internalized Cholesterol in Normal and Mutant Cell Preparations**—This documented excessive lysosomal storage and tardy intracellular mobilization of cholesterol in Niemann-Pick C fibroblasts suggested that LDL uptake by the mutant cells resulted in sequestration of exogenously derived cholesterol within a metabolically silent or trapped pool. In order to explore this possibility, advantage was taken of the reported in vitro modulations of cholesterol ester formation through direct alterations of cholesterol within membranes that contain acyl-CoA:cholesterol acyltransferase (19, 20), the enzyme responsible for intracellular
cholesterol esterification (21). In mutant Niemann-Pick C cells, the availability of cholesterol for interaction with acyl-CoA:cholesterol acyltransferase was considered potentially latent because of possible topological hinderances which could be envisioned to block the translocation of sterol to the catalytic site of acyl-CoA:cholesterol acyltransferase. Following in vivo uptake of LDL, subsequent in vitro esterification of internalized cholesterol could be modulated in cell-free extracts of mutant, but not normal, cells by regulating the extent of secondary organelle disruption (Table II). Following LDL uptake, in vitro synthesis of cholesterol \([\text{H}]\)olate from endogenous cholesterol stores could be varied in mutant cell-free extracts from levels below \((<20\%)\) to above \((150\%)\) those of comparably treated control cell extracts by controlling the extent of subcellular organelle disruption. It should also be noted that the relative in vitro deficiency of cholesterol esterification observed in mutant cell-free extracts with intact subcellular organelles corresponded to the relative deficiency of cholesterol esterification observed in situ with intact Niemann-Pick C cells.

In principle, activation of in vitro cholesterol esterification secondary to a disruption of intracellular membranous structures may just as readily reflect latency on the part of acyl-CoA:cholesterol acyltransferase as it could the lack of available cholesterol. Acyl-CoA:cholesterol acyltransferase has been shown to reside normally on the cytoplasmic side of the rough endoplasmic reticulum (22). With regard to the latency of cholesterol esterification in Niemann-Pick C fibroblasts, the evidence strongly favors the existence of a sequestered and metabolically unavailable pool of exogenously derived cholesterol rather than a topologically misplaced acyl-CoA:cholesterol acyltransferase enzyme: (a) the phenotypic abnormalities presented by the Niemann-Pick C mutation reflect not only deficient acyl-CoA:cholesterol acyltransferase catalysis but also deficient down-regulation of two other cholesterol-regulated proteins, the LDL receptor and hydroxymethylglutaryl-CoA reductase (11), (b) the histochemical findings clearly show abnormal sterol accumulation, (c) normal orientation of acyl-CoA:cholesterol acyltransferase on the cytosolic side of the endoplasmic reticulum in mutant cells is supported by the finding that acyl-CoA:cholesterol acyltransferase of mutant cells was as susceptible, in gently disrupted cell homogenates, to proteolytic inactivation by added proteases as the enzyme of normal cell extracts (data not shown).
(d) *in vivo* and *in vitro* cholesterol esterification was normal or even somewhat elevated in mutant cells not cultured with LDL (Table II).

**DISCUSSION**

Type C Niemann-Pick disease appears to represent a newly defined and unique cholesterol storage disorder. The pathogenic abnormalities include major disruptions of intracellular cholesterol processing. In mutant fibroblasts, extracellular LDL is carried by receptor-mediated endocytosis to lysosomes where apparently normal proteolytic and lipolytic processing of the exogenous lipoprotein initially takes place (11). However, the subsequent intracellular fate of the lysosomal cholesterol and the normal cellular responses to cholesterol uptake are compromised by the mutation. A translocation of exogenously derived cholesterol from lysosomes appears deficient. The internalized and sequestered cholesterol of Niemann-Pick C fibroblasts fails to initiate the prompt homeostatic responses that serve to control and to balance intracellular cholesterol levels in normal cells. There is associated with the Niemann-Pick C mutation a tardy down-regulation of the LDL receptor, a delayed suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and a defective stimulation of acyl-CoA:cholesterol acyltransferase expression (11). As would be predicted, these delayed metabolic responses lead to excessive intracellular accumulation of unesterified cholesterol which is primarily stored in lysosomes (Fig. 2).

This defective lysosomal translocation of cholesterol is not only associated with delayed homeostatic responses but also with an impaired enrichment of cholesterol in the plasma membranes of mutant cells (Fig. 3 and Table I). Abnormal intracellular cholesterol sequestration can also be inferred from the finding that additional extensive organellae disruption in cell-free extracts greatly enhances the *in vitro* availability of cellular cholesterol for esterification in cell-free extracts of mutant fibroblasts cultured with LDL (Table II).

The molecular basis for the abnormal lysosomal sequestration of LDL-derived cholesterol in Niemann-Pick C disease is not known. LDL-cholesterol released in lysosomes is thought to reach the endoplasmic reticulum and the Golgi apparatus (23). Saturation of a limited sterol pool within the endoplasmic reticulum presumably initiates the numerous cellular homeostatic responses that enable normal cells to regulate intracellular cholesterol levels. The components of this cholesterol transport process from lysosomes to the endoplasmic reticulum are not known. It has been speculated that active vesicular or carrier-mediated transport may be involved (23).

It is likely that Niemann-Pick C disease will prove to be a useful pathological model for elucidating additional steps of intracellular cholesterol processing. Earlier documentation of induced or genetic perturbations of the LDL pathway at the lysosomal step has been limited to the observations that blocked hydrolysis of LDL cholesterol esters leads to lysosomal accumulation of unhydrolyzed esters and retarded homeostatic responses (24–27). The Niemann-Pick C mutation clearly affects a step subsequent to hydrolytic lysosomal processing (11). Accumulation of unesterified cholesterol within the lysosomes of mutant cells begins to exceed the levels found in normal cells as early as 2 h after initiation of LDL uptake, and by 24 h an extensive lysosomal cholesterol pool

### TABLE I
Cellular distribution of unesterified cholesterol in normal and Niemann-Pick C fibroblasts

Stock normal and mutant Niemann-Pick C fibroblasts were harvested and seeded at a density of 9 × 10⁶ cells in 850-cm² roller culture bottles with 100 ml of Eagle's minimal essential medium, 2 mM glutamine, and 10% fetal bovine serum for 2 days. The culture medium was replaced with fresh McCoy's medium with 10% lipoprotein-deficient human serum and 2 mM glutamine for 4 days. This medium was replaced with fresh medium + 50 μg/ml of LDL protein/ps and monolayers incubated an additional 24 h. Roller bottles were rinsed three times with 20 ml of PBS and subsequently harvested with 10 ml of 0.05% trypsin in PBS for 5 min at 37 °C. Cell suspensions were subsequently pelleted at 700 × g for 5 min and cells washed three times with 10 ml of PBS. Cell pellets (4–5 mg of protein) were suspended in 1.0 ml of PBS and kept on ice for subsequent analytical procedures described under "Experimental Procedures." The data point is the average of two separate cell cultures.

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>LDL addition</th>
<th>Unesterified cholesterol levels</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 μg/ml/24 h</td>
<td>Total in cell</td>
<td>Plasma membrane</td>
</tr>
<tr>
<td>Normal (3)</td>
<td>--</td>
<td>55</td>
<td>13</td>
</tr>
<tr>
<td>Mutant (3)</td>
<td>--</td>
<td>73</td>
<td>13</td>
</tr>
<tr>
<td>Normal (2)</td>
<td>+</td>
<td>123</td>
<td>57</td>
</tr>
<tr>
<td>Mutant (2)</td>
<td>+</td>
<td>167</td>
<td>28</td>
</tr>
</tbody>
</table>

* No additional LDL.

### TABLE II
Accessibility of cellular cholesterol to *in vitro* and *in vivo* esterification in normal and Niemann-Pick C fibroblasts

Stock cell cultures were harvested and seeded at 9 × 10⁶ cells in 850-cm² roller bottles and at 3 × 10⁶ cells in 25-cm² flasks in 100 and 7 ml, respectively, of Eagle's minimal essential medium + 10% fetal bovine serum for 2 days. The cultures were depleted of cellular cholesterol by culturing in McCoy's medium + 10% FBS for 4 days. Medium was replaced with fresh medium ± 50 μg LDL protein/ml for 12 h. To the smaller 25-cm² culture flasks, 0.012 ml of 6 mM [3H]oleate (200 dpm/pmol) in 14% acid-free bovine serum albumin was added for the last 2 h of the incubation. These particular cultures were washed, harvested, and subsequently analyzed for *in vitro* cholesterol [3H]oleate synthesis by lipid extraction and thin layer chromatography (5). The larger cell cultures were also incubated ± LDL (50 μg/ml) for 12 h and subsequently washed and harvested as described in Table I. These cell suspensions were analyzed for unesterified cholesterol levels and *in vitro* cholesterol [3H]oleate formation as described under "Experimental Procedures." The determinations represent the average of two separate cell cultures.

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>LDL co-culture</th>
<th>Cholesterol [3H]oleate synthesis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>50 μg/ml/12 h</td>
<td>[3H]oleate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nmoi/mg protein</td>
</tr>
<tr>
<td>Normal (2)</td>
<td>--</td>
<td>20</td>
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<td>Mutant (2)</td>
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<td>30</td>
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<tr>
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<td>40</td>
</tr>
<tr>
<td>Mutant (2)</td>
<td>+</td>
<td>90</td>
</tr>
</tbody>
</table>

* No additional LDL.
has formed (Fig. 1). Previous studies have shown that the total cellular accumulation of LDL cholesterol in these mutant cells does not exceed that of normal cells during the first 6 h of lipoprotein uptake (8). Consequently, excessive storage of cholesterol within lysosomes of the affected cells at this early phase of lipoprotein uptake would suggest a delay in the translocation of cholesterol from lysosomes to further intracellular sites of distribution rather than over active endocytic uptake.

The relationship between lysosomal storage and deficient intracellular mobilization of cholesterol in Niemann-Pick C fibroblasts suggests several possible disruptive mechanisms. Excessive lysosomal cholesterol accumulation may represent a primary lesion at the lysosome itself. The diffusion of hydrolyzed metabolites from lysosomes is often under the control of carrier-mediated processes (28). Lysosomal accumulation of cystine in cystinosis (29) and sialic acid in Salla disease (30) are examples of blocked translocations of metabolic products from loaded lysosomes.

On the other hand, it is also possible that lysosomal cholesterol accumulation simply reflects the capacity and availability of lysosomes to store cholesterol when they are called upon to do so because of some more distal primary block. Potential primary post-lysosomal abnormalities could include deficient sterol carrier proteins or lesions in membrane interactions which normally serve to transport cholesterol to specific target sites. A partial and temporary manifestation of excessive cholesterol storage and deficient sterol transport in heterozygous mutant Niemann-Pick C fibroblasts during only the early active phase of LDL uptake (8) tends to favor the possibility that these cells were temporarily oversaturated and partially deficient in some carrier or receptor-mediated translocation process. It should also be noted that even with homozygous mutant fibroblasts, errors of cholesterol processing reflect delayed or tarry cellular responses rather than absolute deficiencies (11). Whether the recovery toward normal responses represents a "leaky" mutation or secondary pathways of lysosomal cholesterol processing remains to be established. The heterogeneity in the clinical presentations of Niemann-Pick C patients (8) and the variability noted in the cholesterol processing deficiencies of different Niemann-Pick C cell lines (6, 7, 10) suggests that such considerations may be pertinent for a fuller understanding of the clinical and molecular pathogenesis of this disease.

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REFERENCES