Differential accumulation of cholesterol in Golgi compartments of normal and Niemann-Pick Type C fibroblasts incubated with LDL: a cytochemical freeze-fracture study

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Abstract Cholesterol accumulation in the Golgi of normal and Niemann-Pick Type C (NP-C) fibroblasts was shown by freeze-fracture electron microscopy using filipin as a probe for unesterified cholesterol. The specific distribution of cholesterol within individual Golgi compartments could be examined because membrane cholesterol forms complexes with filipin that are visible as membrane deformations (pits and protuberances) in freeze-fracture replicas. The density of filipin-cholesterol deformations, quantitated for *cis, medial,* and *trans* Golgi cisternae and *trans* Golgi vacuoles, was shown to increase in a *cis* to *trans* direction. After addition of low density lipoproteins (LDL) to cultured fibroblasts for 24 h, the cholesterol content increased within specific compartments of the Golgi. Normal cells showed an increase in filipin-cholesterol deformations in membranes of *cis/medial* cisternae and *trans* Golgi vacuoles, whereas NP-C cells showed only an increase in membranes of *trans* Golgi cisternae. LDL uptake by cells appears to induce a disparate cholesterol enrichment of Golgi compartments of normal and mutant cells. The ability of cells to process endocytosed cholesterol may in part depend on modulation of cholesterol-enriched membrane transport through the Golgi, a function which appears to be defective in NP-C cells.—**Coxey, R. A., P. G. Pentchev, G. Campbell, and E. J. Blanchette-Mackie.** Differential accumulation of cholesterol in Golgi compartments of normal and Niemann-Pick Type C fibroblasts incubated with LDL: a cytochemical freeze-fracture study. *J. Lipid Res.* 1993. **34:** 1165–1176.

Supplementary key words unesterified cholesterol transport • low density lipoprotein • filipin

Exogenous cholesterol is provided to cells primarily through endocytic uptake of circulating cholesteryl ester-rich lipoproteins via the LDL receptor-mediated pathway (1). Endocytosed lipoproteins are transferred to lysosomes where hydrolysis of cholesteryl esters releases free cholesterol for subsequent transport to the plasma membrane as well as other cellular organelles. The intracellular mobilization of this LDL-derived cholesterol from lysosomes is important in maintenance of cellular cholesterol homeostasis. Movement of cholesterol from lysosomes is associated with stimulation of cholesteryl ester synthesis, and suppression of de novo cholesterol synthesis and LDL receptor activity. Excess cholesterol is reesterified in the endoplasmic reticulum by acyl coA:cholesterol acyltransferase and resulting cholesteryl esters are stored in intracellular lipid droplets. When circulating lipoproteins are decreased, cells use endogenous cholesterol from those cholesteryl ester storage pools or from de novo synthesis.

Cholesterol transport in cells appears to be a regulated process as reflected by the heterogeneous distribution of cholesterol in membranes. The highest concentration of cholesterol is found in plasma membrane (2) and the remainder is distributed in decreasing amounts between lysosomal, Golgi, and endoplasmic reticulum membranes (3, 4). The means by which exogenously derived cholesterol leaves lysosomes, the pathways by which cholesterol moves intracellularly, and the mechanisms responsible for maintaining cholesterol heterogeneity in cell membranes is poorly understood. Possible mechanisms involved in movement of lipid molecules between intracellular membranes include vesicular budding, organelle fusion, protein transport, and lateral flow in the plane of membranes (5–7).

Abbreviations: NP-C, Niemann-Pick Type C; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum; PBS, phosphate-buffered saline.

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Niemann-Pick Type C (NP-C) disease, a human autosomal-recessive cholesterol storage disorder, offers a valuable model for the study of intracellular cholesterol transport. LDL uptake by NP-C fibroblasts is associated with delayed cholesterol homeostatic responses. These delayed homeostatic responses have been shown to be due to an abnormal sequestration and accumulation of cholesterol in lysosomes (8). Recently we demonstrated cholesterol accumulation in Golgi of both normal and NP-C fibroblasts incubated with LDL, suggesting that endocytosed lipoprotein cholesterol may be transported from lysosomes to other cellular destinations through the Golgi (9). This initial demonstration of Golgi involvement in intracellular cholesterol transport was documented with fluorescence microscopy using filipin as a probe for unesterified cholesterol. In this particular study, we found also that NP-C fibroblasts accumulate cholesterol in Golgi earlier than normal fibroblasts, suggesting that the cholesterol transport defect in these cells may reflect, in part, a Golgi lesion (9).

The Golgi is a complex polarized organelle consisting of both a *cis* and *trans* side, containing compartments with functionally different capacities for directing cellular components (10). Although our previous fluorescence studies in fibroblasts showed that the *trans* side of the Golgi was enriched in cholesterol after LDL uptake (9), we could not resolve whether other Golgi compartments were involved. In the current study, we have begun to dissect the role of Golgi compartments in intracellular cholesterol transport. Filipin complexes with unesterified cholesterol in membranes forming structures which are visible with freeze-fracture electron microscopy (11). Quantitation of these complexes allowed us to determine the relative location of cholesterol in the membranes of various Golgi compartments. The reported findings and our current understanding of Golgi membrane movement suggest that exogenously derived cholesterol may be transported through the Golgi in reaching intracellular locations and that cholesterol transport from *trans* Golgi cisternae is defective in NP-C fibroblasts.

MATERIALS AND METHODS

Cell cultures

Normal and NP-C fibroblasts were derived from volunteers and confirmed patients of the Developmental and Metabolic Neurology Branch, National Institutes of Health, under the guideline approved by the NIH clinical research committee. Fibroblasts (3–15 passages) were cultured in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (HyClone Laboratories, Inc., Logan, UT), 1% nonessential amino acids, 2 mM glutamine, and 100 units penicillin-streptomycin/ml in humidified 95% air and 5% CO_2 at 37°C. Stock fibroblasts were incubated in standard EMEM culture medium containing 10% lipoprotein-deficient bovine serum (LPDS) for 8 days, harvested by trypsinization, and plated at a density of 20 × 10^3 cells per 9.5 cm^2, either in 150-mm glass culture dishes for freeze-fracture and fluorescence studies or in Lab-Tek plastic microscope slide chambers (Miles Scientific, Naperville, IL) for fluorescence and thin-sectioning studies. After 2 days, cells were incubated with or without LDL (50 μg protein/ml) for 24 h before cells were prepared for microscopic analysis. LPDS and human low density lipoprotein (LDL) were purchased from Advanced Biosciences Laboratories, Rockville, MD.

Concurrent fluorescent cytochemical staining of cholesterol and immunocytochemical staining of lysosomes

Cells in plastic slide chambers and in 150-mm glass culture dishes were washed with PBS, pH 7.4, fixed in 3% (wt/vol) paraformaldehyde containing 0.2% glutaraldehyde for 30 min at room temperature, and then washed with PBS. All subsequent steps, antibody incubations and washes, were carried out in PBS that contained 50 μg/ml filipin (Polysciences Inc., Warrington, PA) and 125 μg/ml goat IgG (Jackson Immunochemicals, Avondale, PA). Cells were incubated at room temperature for 30 min in 0.15% glycine–PBS to quench free aldehyde groups and then for 60 min with a monoclonal antibody to human lysosomal membrane protein (12) at a 1:4 dilution in PBS. Cells were washed in PBS to free unbound antibody and then incubated for 60 min with rhodamine-conjugated affinity-purified goat anti-rat IgG (Jackson Laboratories, Avondale, PA) at a 1:40 dilution in PBS. Cells were washed in PBS, mounted in *p*-phenylenediamine glycerol, and viewed with a Leitz fluorescence microscope by using excitation filters, BP-350-410 for filipin and BP-530-560 for rhodamine.

Electron microscopic analysis of fibroblast cultures (thin sections and freeze-fracture replicas)

Cells cultured in microscope slide chambers were fixed in Karnovsky's fixative (13) for 60 min, washed with 0.2 M sodium cacodylate buffer, pH 7.4, fixed in 2% (wt/vol) OsO_4 in 0.2 M sodium cacodylate buffer for 2 h at 4°C, dehydrated in a graded series of ethanols, and embedded in LX112 resin (Polyscience Inc, Warrington, PA). After hardening in a 60°C oven, the resin-embedded cells were removed from the plastic slide and thin-sectioned for transmission electron microscopy.

Cells cultured in 150-mm glass culture dishes were fixed in Karnovsky's fixative containing filipin for 30 min at room temperature. Filipin was dissolved in DMSO at 25 mg/ml which was then diluted to 65 μg/ml with fixative. Fibroblasts were scraped from random areas of the culture dishes, pelleted in fresh fixative in microfuge tubes

(1 min at 15,000 rpm), cryoprotected in 30% glycerol in 0.2 M sodium cacodylate (pH 7.4), and immediately frozen in Freon cooled with liquid nitrogen. Freeze-fracture replicas of pellets were prepared in a Balzers model BAF 301 freeze-etch device.

Thin sections and replicas were viewed with a Philips 400 transmission electron microscope, equipped with a goniometer stage. Each area of the Golgi was photographed twice at a tilt of 6 degrees to produce stereo images (see below).

Identification of Golgi compartments

In cultured human fibroblasts, Golgi consisted of discrete stacks of three to five cisternae (*cis, medial,* and *trans* cisternae) and *trans* Golgi vacuoles. *Trans* Golgi vacuoles were circular structures 100–350 nm in diameter and were located adjacent to the *trans* side of the cisternal stack (14). Therefore, cisternae within 200 nm of *trans* Golgi vacuoles were classified as *trans* cisternae. When Golgi stacks consisted of more than three cisternae, the two cisternae adjacent to *trans* Golgi vacuoles were classified as *trans* cisternae and those more distal were classified as *medial* and *cis* cisternae. The *cis* side of the Golgi consists of *cis* and *medial* cisternae while the *trans* side of Golgi consists of *trans* cisternae and *trans* Golgi vacuoles. The few vacuoles found in the vicinity of the Golgi stack with irregular shapes and diameters larger than 350 nm were classified as lysosomes.

Freeze-fracture cytochemistry

Freeze-fracture cytochemistry, with filipin as a probe to localize unesterified cholesterol, was used to study the distribution of cholesterol in compartments on the *cis* and *trans* side of the Golgi stack in both normal and NP-C fibroblasts. Filipin complexes with unesterified cholesterol in membrane bilayers (15) to induce deformations detectable by freeze-fracture as pits or protuberances (20–25 nm in diameter) on inner, hydrophobic faces (fracture faces) of cytoplasmic and lumenal leaflets of membranes (16, 17). Because of the randomness of the fracture plane through Golgi membranes, the various sized membrane areas that were exposed ranged from 0.003 μm^2 to 0.415 μm^2. The mean membrane area for each Golgi compartment ranged from 0.016 μm^2 to 0.075 μm^2. Each membrane area exposed by the fracture plane was considered a single observation. The number of pits and protuberances were counted in each area of membrane leaflet and the density of filipin-cholesterol deformations was computed per square micron of membrane surface. The periphery of each area of membrane face, roughly perpendicular to the viewing axis, was traced and recorded on a "Summagraphics" graphic tablet (Summagraphic Corporation, Seymour, CT) connected to an "Imagepro II" IBM computer program (Media Cybernetics, Inc., Silver Spring, MD). Stereo-paired electron micrographs were taken (at 6 degrees of tilt) of all Golgi membranes to produce three-dimensional images, which aided in evaluating the flatness of membrane leaflets. It was necessary also to view each Golgi image in stereo to determine, with certainty, whether lumenal and cytoplasmic membrane leaflets belonged to *cis, medial,* or *trans* cisternae. The density of filipin-cholesterol membrane deformations was measured for *cis, medial,* and *trans* Golgi cisternae and *trans* Golgi vacuoles. Data for *cis/medial* cisternae were grouped together for analysis.

Statistical analyses of filipin-cholesterol deformations in Golgi compartments

Four groups of fibroblasts were analyzed in this study; normal and NP-C fibroblasts grown in lipid depleted media, and normal and NP-C fibroblasts incubated with LDL for 24 h. We compared the density of filipin-cholesterol deformations in membrane areas of Golgi compartments (*cis/medial* cisternae, *trans* cisternae, and *trans* Golgi vacuoles) within individual as well as between the various groups of fibroblasts. Within a single Golgi compartment, there was a high degree of variability in the density of filipin-cholesterol deformations in areas of membrane leaflets. Some areas of membrane leaflets had no deformations, while other areas were packed so tightly with deformations (saturated) that the boundaries of pits and protuberances were not discernable and therefore could not be counted as separate entities. Saturated membrane areas were arbitrarily assigned a density of 500 deformations/μm^2 of membrane (a value greater than the highest density of 380 deformations/μm^2 which was clearly discernable). Membrane areas in which density of deformations could be determined were classified as partially saturated. Comparisons were made within and between experimental groups using all Golgi membranes, which includes areas without deformations and areas partially saturated and saturated with deformations (see Table 1). Comparisons were also made between experimental groups and between Golgi compartments using density of filipin-cholesterol deformations of partially saturated membranes, percentage of membrane area partially saturated with deformations, and percentage of membrane area saturated with deformations (see Table 2).

The data were analyzed by nonparametric methods using the Kruskal-Wallis test (18) to determine whether the deformation densities of all Golgi membranes or of partially saturated membrane differed among the three Golgi compartments. If the density of membrane deformations was judged to differ significantly among the three Golgi compartments, Wilcoxon tests were performed, preserving the overall Type I error (19) to determine which pairs of compartmental densities differed. For each Golgi compartment, statistical comparisons were made between normal fibroblasts incubated with or without LDL and between NP-C fibroblasts incubated with or

without LDL. Also, for each Golgi compartment, statistical comparisons were made between normal and NP-C fibroblasts incubated without LDL and between normal and NP-C fibroblasts incubated with LDL. For the proportion of areas saturated with deformations, overall exact contingency table tests were performed to decide whether there were differences, followed by Fisher exact tests to conclude pairwise differences. To guard against false positive differences, all comparisons and reported *P* values were adjusted using a Bonferroni correction factor (20) of four, as they were made among four experimental treatments (normal or NP-C fibroblasts incubated with or without LDL). In addition, the distribution of pits in lumenal and cytoplasmic leaflets of Golgi membrane bilayers of normal fibroblasts was quantified for each of the experimental groups.

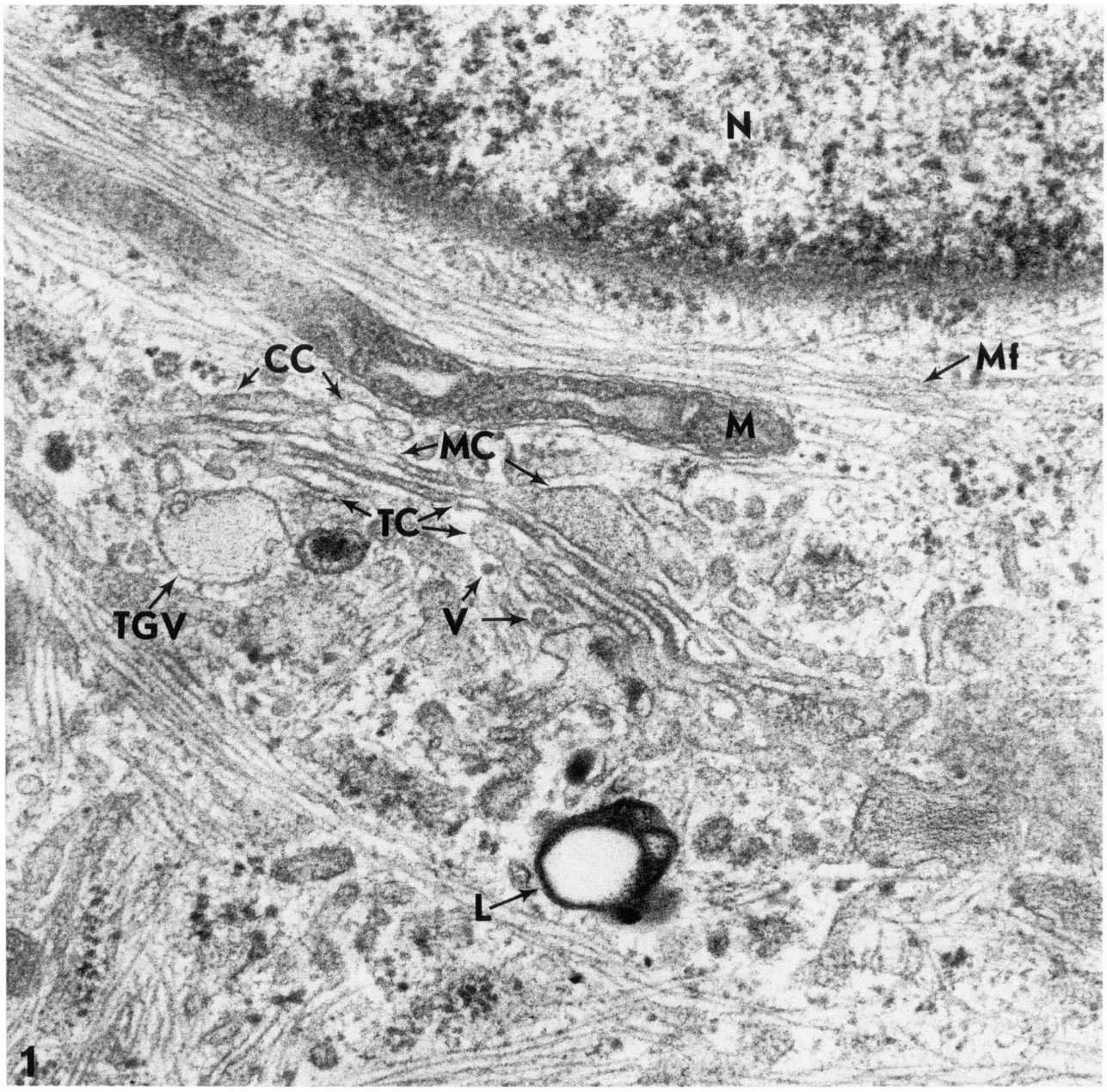

Fig. 1. Electron micrograph of a thin section of a Golgi stack in a cultured human fibroblast incubated with LDL for 24 h. This Golgi stack consists of four cisternae, small vesicles (v) 20–100 nm in diameter, and a *trans* Golgi vacuole (TGV). The two cisternae proximal to the *trans* Golgi vacuole are *trans* (TC) cisternae, while the cisternae more distal to the *trans* Golgi vacuole are *medial* (MC) and *cis* (CC). Lysosomes, L; mitochondria, M; nucleus, N; microfilaments, Mf. 68750 × magnification.

RESULTS

The Golgi complex in cultured fibroblasts consists of stacks of cisternae, vesicles, and vacuoles. With light microscopy and wheat germ agglutinin staining, the Golgi complex was usually seen in a paranuclear position in both normal and NP-C fibroblasts although the size and shape of the Golgi conformed somewhat to cellular size and shape, i.e., elongated Golgi in elongated cells and round, more compact Golgi in spherical cells. Electron microscopy revealed that each Golgi stack consisted of three to five cisternae, small vesicles (20–100 nm) associated with all cisternae, and vacuoles (100–350 nm) located on one side of the cisternal stack (**Fig. 1**). These vacuoles were similar in size and location to structures previously identified in cultured fibroblasts as exocytic *trans* Golgi vacuoles by wheat germ agglutinin staining and immunostaining for fibronectin (14) and they marked

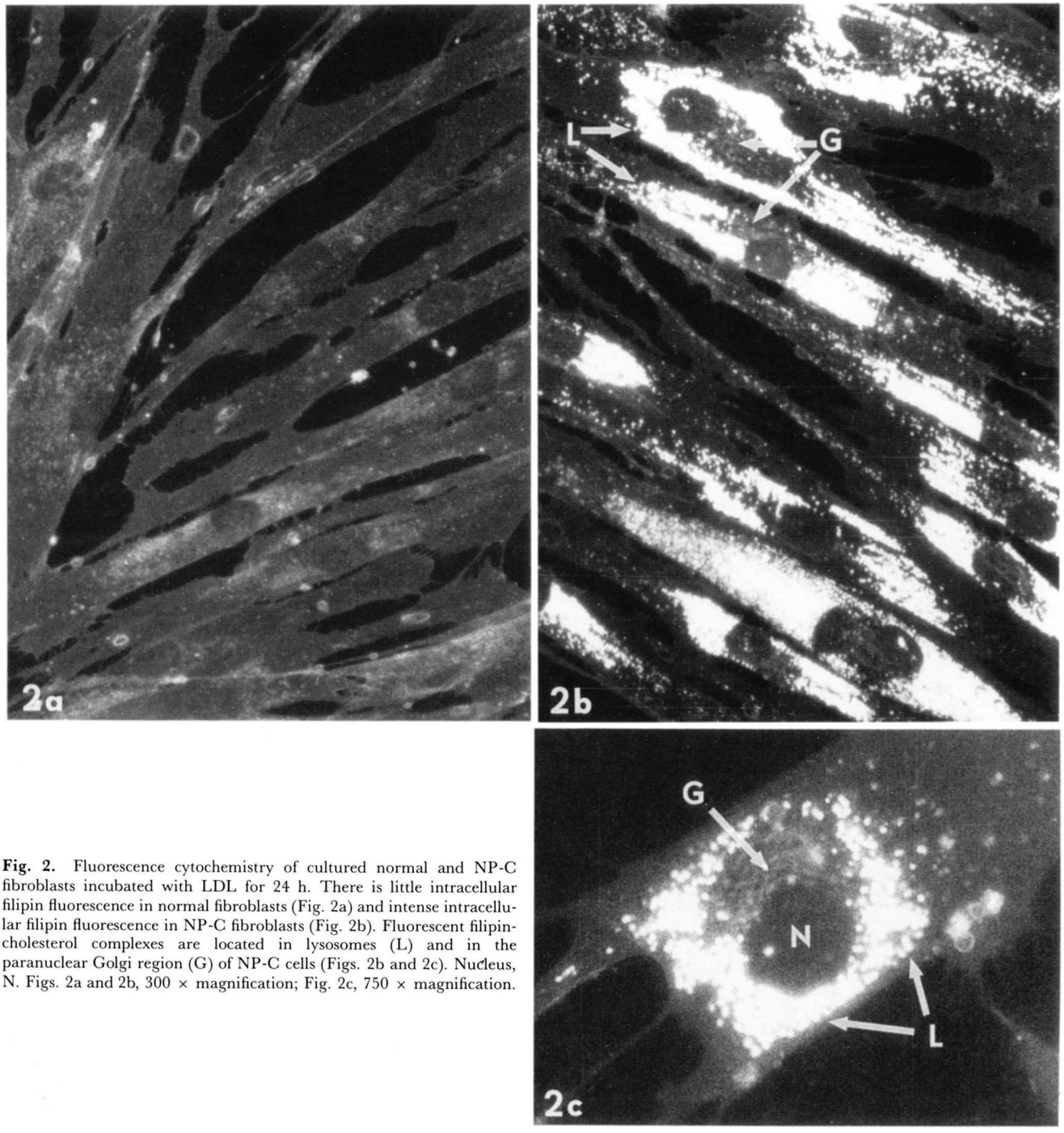

Fig. 2. Fluorescence cytochemistry of cultured normal and NP-C fibroblasts incubated with LDL for 24 h. There is little intracellular filipin fluorescence in normal fibroblasts (Fig. 2a) and intense intracellular filipin fluorescence in NP-C fibroblasts (Fig. 2b). Fluorescent filipin-cholesterol complexes are located in lysosomes (L) and in the paranuclear Golgi region (G) of NP-C cells (Figs. 2b and 2c). Nucleus, N. Figs. 2a and 2b, 300 × magnification; Fig. 2c, 750 × magnification.

the *trans* side of the Golgi. Cisternae within 200 nm of *trans* Golgi vacuoles were classified as *trans* cisternae. The morphology of individual Golgi stacks appeared similar in normal and NP-C fibroblasts and was not altered in cells incubated for 24 h with LDL.

We had previously shown that incubation of NP-C fibroblasts with LDL resulted in accumulation of cholesterol in the Golgi complex within 2 h, while in normal cells, similar cholesterol enrichment was not observed until 24 h (9). Extensive cholesterol accumulation was present also in lysosomes in NP-C fibroblasts but not in normal fibroblasts (8, 21). In the present study, filipin fluorescence was used to monitor the lysosomal and Golgi accumulation of cholesterol in samples prior to further processing for freeze-fracture electron microscopic analysis. After a 24-h incubation with LDL, normal cells showed less filipin staining of lysosomes (**Fig. 2a**) than NP-C cells (Figs. 2b and 2c). The cholesterol-laden lysosomes of NP-C cells, located in a paranuclear position in the cells (Fig. 2b) were large, irregularly shaped organelles, with a distinctive, tightly packed multilamellar core component (**Fig. 3b and inset**). Lysosomes of normal and NP-C cells (Fig. 3a) incubated without LDL were, however, similar in structure. They contained mainly electron lucent cores, or small vesicles and remnants of digested organelles, but not the distinctive multilamellar core component of cholesterol enriched NP-C lysosomes.

Freeze-fracture cytochemistry was used to further determine the distribution of cholesterol within membranes of Golgi compartments. Stereo-paired electron micrographs were viewed to determine whether membrane leaflets belonged to *cis, medial,* and *trans* cisternae and to determine whether membrane leaflets were roughly perpendicular (flat) to the viewing axis (**Fig. 4**). Filipin-cholesterol complexes formed deformations in membrane bilayers which were quantitated in membrane areas of *cis, medial,* and *trans* cisternae and *trans* Golgi vacuoles. For quantitation, *cis* and *medial* cisternal membranes are grouped together and *trans* cisternae and *trans* Golgi vacu-

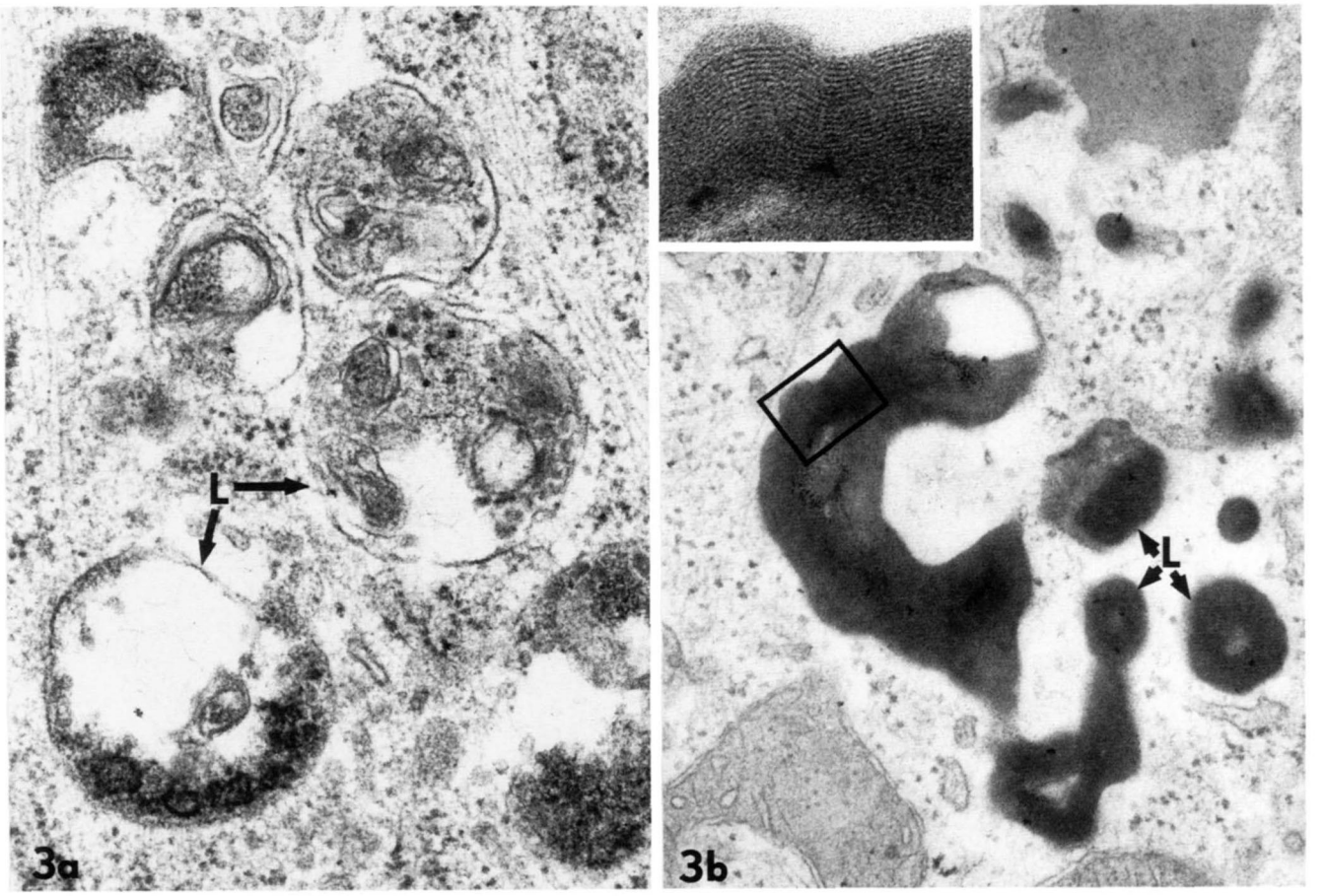

Fig. 3. Electron micrographs of thin section of lysosomes in NP-C cells incubated without (Fig. 3a) and with (Fig. 3b) LDL for 24 h. Lysosomes (L) of NP-C cells incubated without cholesterol have largely electron lucent cores with some internal content of small vesicles and fragments of organelles (Fig. 3a). Lysosomes of NP-C cells incubated with LDL for 24 h are irregular in shape and have a large, tightly packed multilamellar core component with a periodicity of 43 Å (Fig. 3b and higher magnification in inset). Figs. 3a and 3b, 54,000 × magnification; inset 160,000 × magnification.

Fig. 4. Freeze-fracture stereo-paired electron micrographs of a Golgi stack in a cultured normal human fibroblast showing the three-dimensional image of membrane leaflets of Golgi cisternae and their orientation to the viewing axis. The *cis* cisterna (CC) curves underneath the Golgi stack at the bottom of the micrograph revealing portions of its cytoplasmic (c) and lumenal (l) leaflets. The arrowheads point to aqueous space. *Trans* Golgi vacuoles, TGV; medial cisterna, MC; *trans* cisterna, TC. 55000 × magnification. Larger magnification is shown in Fig. 5a.

oles are presented separately. The density of deformations in membrane areas varied to include membrane areas without deformations, as well as partially saturated ($<500/\mu m^2$) and saturated ($>500/\mu m^2$) areas. The results are expressed, in **Table 1,** as density of filipin-cholesterol deformations of Golgi membrane for each Golgi compartment and entire Golgi and, in **Table 2,** as density of filipin-cholesterol deformations of partially saturated membrane and percentage of saturated membrane for each Golgi compartment. These analyses showed significant differences in membrane deformations between Golgi compartments of normal and NP-C cells incubated with and without LDL.

Filipin-cholesterol deformation density was lower in membranes of *cis/medial* cisternae and higher in *trans* cisternae of both normal and mutant cells incubated with and without LDL (Table 1). This increasing gradient of filipin-cholesterol deformations from the *cis* to *trans* side of the Golgi is similar to the gradient reported for filipin-cholesterol deformations in Golgi cisternae in pancreatic acinar cells (22). We found that LDL uptake induced an increase in the density of filipin-cholesterol deformations in Golgi membranes (Table 1) and that the compartmental distribution of these deformations in normal and NP-C cells differed (Tables 1 and 2).

In both normal and NP-C fibroblasts incubated with LDL for 24 h, the density of filipin-cholesterol deformations of Golgi membranes for the entire Golgi tripled in normal fibroblasts and at least doubled in NP-C fibroblasts (Table 1). The increased filipin-cholesterol binding in Golgi in response to LDL uptake occurred in membranes of *cis/medial* cisternae and *trans* Golgi vacuoles in normal cells and, in contrast, in membranes of *trans* Golgi cisternae in NP-C cells (Tables 1 and 2). The increase in cholesterol of *cis/medial* cisternae of normal cells is shown by analyses of Golgi membranes (Table 1) or partially saturated membranes (Table 2). Enrichment of cholesterol in *trans* Golgi vacuoles of normal cells was noted by the increase in percentage of saturated membranes (Table 2 and **Figs. 5a and 5b**). In contrast, NP-C cells showed an increase in filipin-cholesterol deformations in membranes of *trans* Golgi cisternae in response to LDL uptake which was not seen in normal cells and contained fewer filipin-cholesterol deformations in *cis/medial* cisternae than

TABLE 1. Effect of LDL uptake on distribution and density of filipin-cholesterol deformations (FCD) in Golgi membranes of normal and NP-C fibroblasts

	Membrane Leaflets			
	Density of FCD in Golgi membranes (number/μm^2 ± SEM)			
	Normal		NP-C	
Golgi Compartments	− LDL	+ LDL	− LDL	+ LDL
cis/medial cisternae	4 ± 1	18 ± 4[b,c]	2 ± 2	3 ± 2
trans cisternae	38 ± 11[a]	107 ± 32	41 ± 23	224 ± 45[d]
trans Golgi vacuoles	71 ± 19	215 ± 49	99 ± 33	65 ± 21
Golgi	31 ± 6	107 ± 21[e]	41 ± 13	97 ± 20[f]

On day 2, some cell monolayers received 50 μg protein/ml LDL. After further incubation for 24 h, cells were fixed in the presence of filipin and processed for freeze-fracture analysis. Values given represent a set of normal and NP-C cells incubated with LDL under the same conditions and harvested at the same time for freeze-fracture analysis. Values given represent pooled data for P- and E-face membranes. N, total number of areas of P- and E-face membrane leaflets examined for: *cis/medial* cisternae (C/CM), *trans* cisternae (TC), *trans* Golgi vacuoles (TGV), and Golgi (G). Normal, − LDL (59,34,33,126); Normal, + LDL (23,24,19,66); NP-C, − LDL (29,23,20,72); NP-C, + LDL (28,25,17,70). Golgi, average density over the three compartments, weighted by N.

[a]Different from *cis/medial* cisternae of normal cells incubated without LDL, $P < 0.01$. Within each of the other experimental treatments, the *trans* cisternae is different from the *cis/medial* cisternae (N + LDL, $P < 0.02$; NP-C − LDL, $P < 0.05$; NP-C + LDL, $P < 0.001$).

[b]Different from *cis/medial* cisternae of normal cells incubated without LDL, $P < 0.01$.

[c]Different from *cis/medial* cisternae of NP-C cells incubated with LDL, $P < 0.05$.

[d]Different from *trans* cisternae of NP-C cells incubated without LDL, $P < 0.05$.

[e]Different from Golgi of normal cells incubated without LDL, $P < 0.001$.

[f]Different from Golgi of NP-C cells incubated without LDL, $P < 0.05$.

normal cells (Table 1). NP-C cells contained more membrane areas saturated with filipin-cholesterol deformations in *trans* cisternae than in either *trans* Golgi vacuoles or *cis/medial* cisternae (Table 2 and **Figs. 6a and 6b**).

Analysis of the distribution of pits between the lumenal and cytoplasmic leaflets of Golgi membrane bilayers of normal cells showed more pits in the lumenal leaflet than the cytoplasmic leaflet (30 ± 7 μm^2 in cells incubated without LDL and 47 ± 8 μm^2 in cells incubated with LDL vs. 8 ± 5 μm^2 in cells incubated without LDL and 2 ± 2 μm^2 in cells incubated with LDL).

TABLE 2. Effect of LDL uptake on distribution and density of filipin-cholesterol deformations (FCD) in partially saturated and saturated Golgi membranes of normal and NP-C fibroblasts

	Filipin-Cholesterol Deformations in Membrane Leaflets					
	Density of FCD in Partially Saturated Membrane (<500/μm^2)		% of Membrane Areas Partially Saturated with FCD		% of Membrane Areas Saturated with FCD (>500/μm^2)	
Golgi Compartments	− LDL	+ LDL	− LDL	+ LDL	− LDL	+ LDL
Normal						
cis/medial cisternae	18 ± 3	35 ± 4[a]	24	52	0	0
trans cisternae	64 ± 17	72 ± 11	59	62	0	0
trans Golgi vacuoles	195 ± 26	179 ± 20	36	32	0	32[b,c]
NP-C						
cis/medial cisternae	32 ± 3	19 ± 6	7	14	0	0
trans cisternae	65 ± 29	100 ± 32	31	44	4	36[d]
trans Golgi vacuoles	185 ± 41	139 ± 27	40	47	5	0

[a]Different from *cis/medial* cisternae of normal cells incubated without LDL, $P < 0.01$.

[b]Different from *trans* Golgi vacuoles of normal cells incubated without LDL, $P < 0.05$.

[c]Different from *cis/medial* cisternae of normal cells incubated with LDL, $P < 0.05$.

[d]Different from *cis/medial* cisternae ($P < 0.005$) and *trans* Golgi vacuoles ($P < 0.02$) in NP-C cells incubated with LDL.

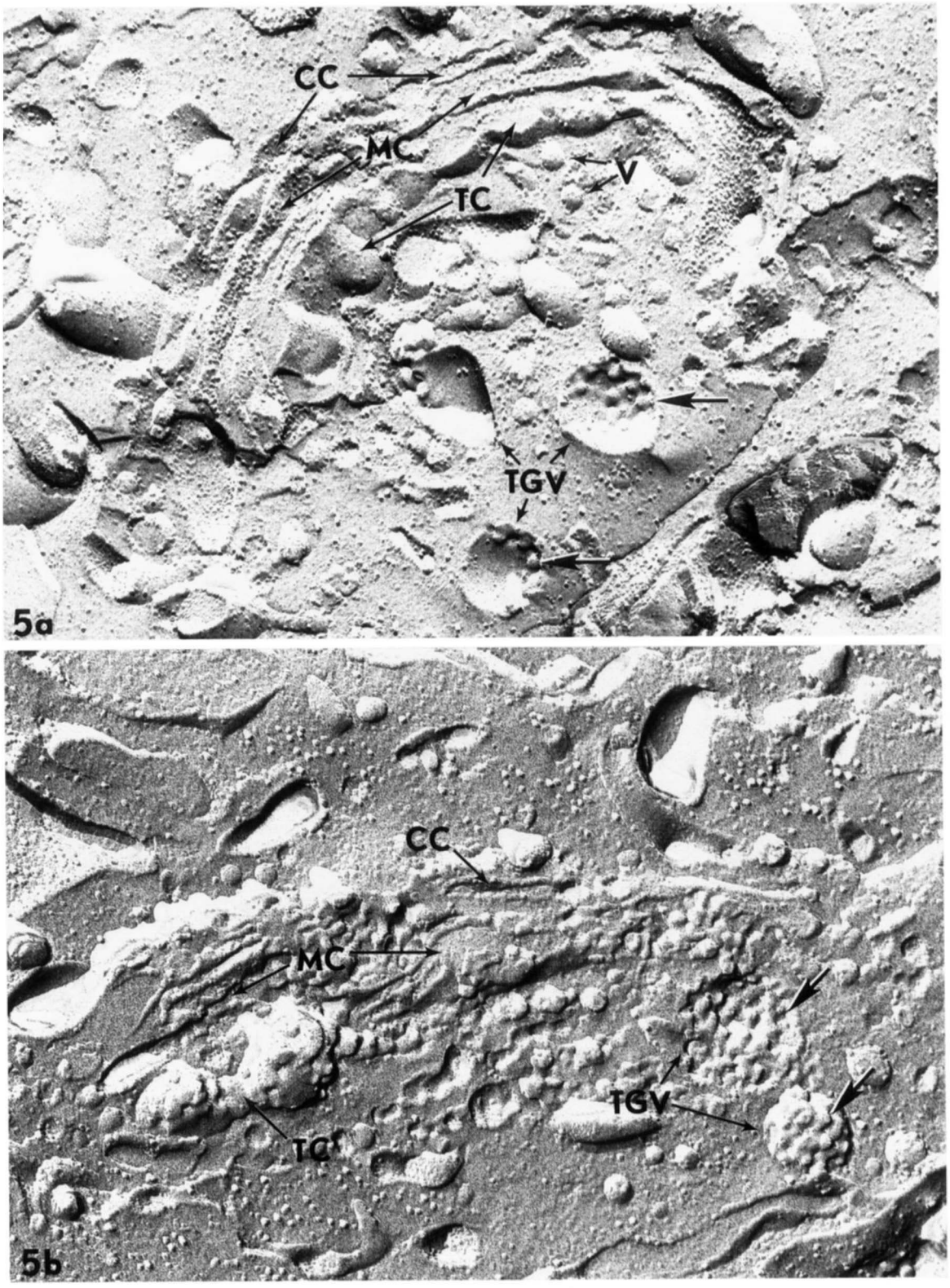

Fig. 5. Electron micrographs of replicas of freeze-fractured, filipin-treated Golgi in normal fibroblasts grown without LDL (Fig. 5a) and with LDL for 24 h (Fig. 5b). Fig. 5a. *Trans* Golgi vacuoles (TGV) have a few filipin-cholesterol deformations (large arrows). Small vesicles (V) are not labeled with filipin. *Cis* cisterna, CC; *medial* cisterna, MC; *trans* cisternae, TC. 74,000 × magnification. Fig. 5b. *Trans* Golgi vacuoles (TGV) of the Golgi are "saturated" with filipin-cholesterol deformations (large arrows) in normal cells incubated with LDL. *Trans* cisterna (TC) has a few filipin-cholesterol deformations. *Cis* cisterna, CC; *medial* cisterna, MC. 77,500 × magnification.

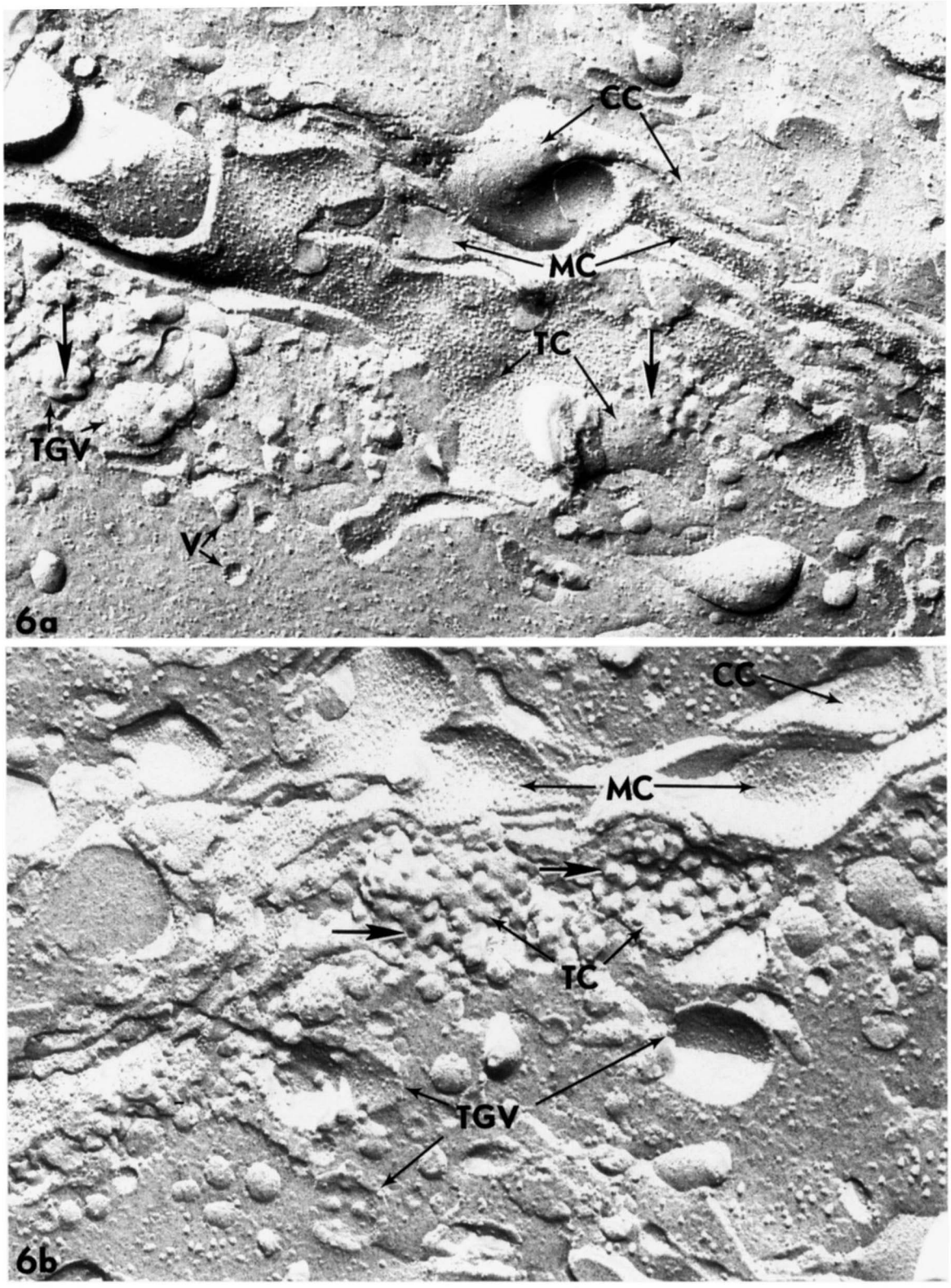

Fig. 6. Electron micrographs of replicas of freeze-fractured, filipin-treated Golgi in NP-C fibroblasts grown without LDL (Fig. 6a) and with LDL for 24 h (Fig. 6b). Fig. 6a. *Trans* cisterna (TC) and *trans* Golgi vacuoles (TGV) have a few filipin-cholesterol deformations (large arrows). Small vesicles (V) are not labeled with filipin. *Cis* cisterna, CC; *medial* cisterna, MC. 74,000 × magnification. Fig. 6b. *Trans* cisterna (TC) are "saturated" with filipin-cholesterol deformations (large arrows) while *trans* Golgi vacuoles (TGV) have a few filipin-cholesterol deformations (large arrows) in NP-C cells incubated with LDL. *Cis* cisterna, CC; *medial* cisterna, MC. 75,000 × magnification.

DISCUSSION

In this study we demonstrate that uptake of low density lipoprotein causes an accumulation of cholesterol in various Golgi compartments of cultured fibroblasts. We found that the concentration of cholesterol in the Golgi of normal and NP-C human fibroblasts increases in a *cis-trans* direction across the Golgi cisternae, similar to that reported for cholesterol concentration across the Golgi in pancreatic acinar cells (22). The *cis-trans* gradient was maintained irrespective of LDL-induced increase in cellular cholesterol levels. The presence of this gradient in the Golgi may serve to modify the cholesterol content of Golgi membranes moving to other intracellular sites and to maintain the cholesterol heterogeneity observed in cellular membranes. Cholesterol concentration is highest in the plasma membrane and there is respectively less in lysosomal and endoplasmic reticulum membranes (1, 2). Membrane of *trans* Golgi containing higher concentrations of cholesterol may selectively move to cholesterol-rich plasma membrane while those of *cis* Golgi may move to similarly less cholesterol-rich membrane such as endoplasmic reticulum.

Although both normal and NP-C fibroblasts showed an increase in density of filipin-cholesterol deformations in Golgi membranes after uptake of LDL for 24 h, there was a difference in the specific compartments of the Golgi that became enriched. In normal fibroblasts, cholesterol increased in both *trans* Golgi vacuoles and *cis/medial* cisternae. Our finding of increased amounts of cholesterol in *trans* Golgi vacuoles in normal fibroblasts suggests a potential means for the intracellular transport of endocytosed cholesterol to the plasma membrane. The possibility exists that cholesterol is transported from lysosomes to the plasma membrane through a Golgi vesicle-mediated process. The rapid movement of *trans* Golgi elements to the cell periphery at a rate of 0.27 μm/sec (23) could carry LDL-derived cholesterol from lysosomes to the plasma membrane, a process reported to have a half-time of several minutes in CHO cells (24). Our observation of cholesterol-enriched *cis/medial* Golgi cisternae in normal cells incubated with LDL may indicate that the *cis* Golgi provides one pathway for cholesterol transport from lysosomes to endoplasmic reticulum for cholesterol esterification. That retrograde membrane movement between Golgi and endoplasmic reticulum occurs has been shown by studies with brefeldin A (25). The accumulation of cholesterol in Golgi in Niemann Pick Type-C fibroblasts after LDL uptake was different. We found cholesterol enrichment in *trans* Golgi cisternae and no evidence of enrichment in *trans* Golgi vacuoles or *cis* Golgi after incubation with LDL. This finding implies that, in mutant cells, cholesterol movement to *trans* Golgi cisternae is not impeded but that subsequent movement from this compartment to *trans* Golgi vacuoles and to *cis/medial* cisternae is impeded. Cholesterol accumulation in *trans* Golgi cisternae due to impaired transport to *trans* Golgi vacuoles and *cis* Golgi could result in defective cholesterol mobilization to the plasma membrane and reduced intracellular cholesteryl ester formation.

In NP-C fibroblasts, defective transport of cholesterol from lysosomes could be explained by defective cholesterol transport through the Golgi. Abnormal accumulation of cholesterol in *trans* Golgi cisternae of NP-C fibroblasts incubated with LDL may reflect sluggish mobilization of cholesterol from this Golgi compartment, secondarily resulting in cholesterol accumulation in lysosomes. In summary, this study suggests that the ability of cells to process cholesterol-rich lipoproteins could, in part, depend on modulation of cholesterol-enriched membrane movement through the Golgi: a Golgi function that appears to be defective in NP-C cells.

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