Basis for rapid efflux of biosynthetic desmosterol from cells

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Abstract **Previous work shows that the efflux of biosynthetic desmosterol from cells is three times more efficient than that of cholesterol. To explain this difference, we labeled CHO-K1 cells with [^{3}H]acetate precursor and measured sterols in the whole cells, plasma membranes and caveolae, and those released to high density lipoprotein (HDL_3). The [^{3}H]desmosterol-to-[^{3}H]cholesterol ratio was similar in the plasma membrane and whole cells but was greater in HDL_3, suggesting that the more efficient efflux of desmosterol is due to more rapid desorption from the plasma membrane. The ratio in caveolae was similar to that in whole cells, arguing against selective delivery of desmosterol to caveolae as an explanation for the more rapid efflux of this sterol. Additionally, to demonstrate that the enhanced release of desmosterol was not due to enhanced intracellular cycling, we made vesicles from CHO-cell plasma membranes labeled with [^{3}H]desmosterol or [^{14}C]cholesterol, and the rapid release of desmosterol was demonstrated in this system. To characterize sterol efflux from a simple lipid bilayer system, we measured the transfer of cholesterol and desmosterol between large unilamellar vesicles (LUV), and found that desmosterol transferred two to three times more rapidly than cholesterol. A similar differential was seen when HDL_3 or low density lipoprotein (LDL) served as the acceptor. These results show that the greater efflux efficiency of biosynthetic desmosterol can be attributed to more efficient desorption from the plasma membrane, and that this difference is a property of the sterols' association with the lipid bilayer. In vivo, the rapid efflux of biosynthetic sterol intermediates, followed by efficient delivery to the liver, may constitute an important mechanism for preventing various types of pathology associated with these materials.—**Phillips, J. E., W. V. Rodrigueza, and W. J. Johnson. **Basis for rapid efflux of biosynthetic desmosterol from cells. *J. Lipid Res.* 1998.** 39: **2459–2470.**

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It is estimated that as much as 90% of the body's cholesterol may be provided by endogenous synthesis in extrahepatic cells and that the majority of sterol released into the pathway of reverse cholesterol transport is derived from this biosynthetic pool (1, 2). Studies using extrahepatic cells in culture have shown that during sterol synthesis, substantial amounts of biosynthetic intermediates "leak" out of the endoplasmic reticulum (ER) and are deposited in the plasma membrane, suggesting that sterol molecules leave the ER before their complete transformation to cholesterol (3). Recently, we reported that in Chinese hamster ovary (CHO) and other nonhepatic cells, newly synthesized desmosterol accumulated to levels comparable to that of newly synthesized cholesterol; additionally, this desmosterol was released several times more efficiently than cholesterol in the presence of HDL and other extracellular sterol acceptors (4). The conversion of plasma-membrane biosynthetic intermediates to cholesterol requires that they return to the ER. However, studies showing the steady accumulation of these intermediates in cells during prolonged incubation (4) and their relatively slow conversion to cholesterol in pulse/chase studies (5) imply that this return process is not particularly efficient. The alternative mechanism for removal of these intermediates from the plasma membrane is efflux and entry into the reverse cholesterol transport pathway (RCT), by which high density lipoprotein (HDL) takes up sterols released from peripheral cell plasma membranes, initiating their transport to the liver for catabolism.

Abbreviations: ADPase, alkaline phosphodiesterase; BBSM, bovine brain sphingomyelin; BSA, bovine serum albumin; CHO, Chinese hamster ovary; CHE, cholesteryl hexadecyl ether; CME, cholesteryl methyl ether; DEAE, diethylaminoethyl; DLP, delipidized serum proteins; GLC, gas–liquid chromatography; HDL, high density lipoprotein; HPLC, high pressure liquid chromatography; IF2, interface 2 from subcellular fractionation; LDL, low density lipoprotein; LUV, large unilamellar vesicle; MEM, minimal essential medium; MWM, molecular weight markers; NABGase, N-acetyl-β-glucosaminidase; PBS, phosphate-buffered saline; PEG, polyethylene glycol; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; POPG, 1-palmitoyl-2-oleoyl-phosphatidylglycerol; PL1, pellet 1 from subcellular fractionation; PNS, post-nuclear supernatant; Shh, sonic hedgehog protein; SLOS, Smith-Lemli-Opitz syndrome.

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The excessive accumulation of sterol intermediates in humans and animals can be very detrimental, leading to such problems as cataracts, aortic lesions, and developmental defects (6–8). It is possible that the efficient efflux of biosynthetic sterol intermediates from the plasma membrane is one of the ways that animals prevent the build-up of these compounds in cells and tissues. In the present study we have investigated the mechanism behind the enhanced efflux of desmosterol. Our objectives were: *1*) to distinguish whether greater efflux was due to more efficient delivery to the plasma membrane or to more rapid release from the plasma membrane; *2*) to establish whether the selective partitioning of desmosterol into caveolae could account for the enhanced release of this sterol; and *3*) to determine whether the faster release of desmosterol could be due to a difference in the association of sterols with the lipid bilayer, by comparing the abilities of cholesterol and desmosterol to transfer out of large unilamellar vesicles (LUV) to a variety of artificial and natural acceptors.

MATERIALS AND METHODS

Materials

CHO-K1 cells were purchased from ATCC. Tissue culture supplies, HPLC and GLC solvents, liquid scintillation vials, and scintillant were purchased from Fisher. Cell culture media and phosphate-buffered saline (PBS) were supplied by Bio-Whitaker. Delipidized serum protein was prepared by the method of Furth et al. (9) using calf serum. Dextran T-500 was purchased from Pharmacia. Sodium [^{3}H]acetate (5.33 Ci/mmol), [1,2-^{3}H]cholesteryl hexadecyl ether ([^{3}H]CHE) (40 Ci/mmol), [1,2-^{3}H]cholesterol (50 Ci/mmol), and [4-^{14}C]cholesterol (57.5 mCi/mmol) were provided by DuPont NEN. 1-Palmitoyl-2-oleoyl-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), bovine brain and egg sphingomyelins were purchased from Avanti Polar Lipids. Cholesterol, desmosterol, protease inhibitors, electrophoresis and Western blotting reagents, chromogenic substrates for both Western blotting and cellular membrane marker assays, chemicals for cholesteryl methyl ether synthesis, polyethylene glycol-8000 (Carbowax-8000), chemicals for plasma-membrane blebbing, fetal bovine serum, and bovine serum albumin (BSA) were purchased from Sigma. Duracryl high tensile acrylamide (a solution of 30% acrylamide and 0.8% bis acrylamide) was supplied by Oxford Glyco Systems. Rainbow molecular weight standards were purchased from Novex and alkaline phosphatase-conjugated molecular weight standards were purchased from Novagen. The primary affinity-purified anti-human polyclonal rabbit caveolin-1 antibody was provided by Transduction Laboratories. The secondary alkaline phosphatase-conjugated goat affinity-purified anti-rabbit IgG antibody was purchased from Pierce. Lipoprotein electrophoresis agarose gels (Paragon Lipogel) and reagents for fixing and staining were purchased from Beckman. Filters (0.1-micron) for LUV extrusion were purchased from Poretics Corporation. PVDF blotting membranes, 0.45-μm 96-well filters, and 0.45-μm filters for tissue culture were purchased from Millipore. Triparanol was a gift from Dr. Ekkehard Bohme (Marion Merrell Dow Inc.).

Methods

Cell culture. All media were supplemented with 50 μg/ml of gentamicin and filtered with a sterile 0.45-micron pore size filter before application to cells. Cells were grown in 100-mm dishes in bicarbonate-buffered Ham's F12 with 7.5% fetal bovine serum (FBS) for 48 h prior to each experiment. For cell fractionation and caveolae isolation experiments, the medium was removed and cells were rinsed with phosphate-buffered saline (PBS) three times. Ham's F12 containing 2 mg/ml of delipidized serum proteins (DLP) and 3% dextran T-500 (w/v) was applied, and this medium was allowed to remain on the cells for an additional 48 h. DLP was used to up-regulate cholesterol synthesis, while dextran was sequestered in lysosomes, making this organellar fraction more dense and easier to separate from plasma membranes (10). Cells were then incubated with [^{3}H]acetate (25 μCi/ml) in HEPES-buffered Ham's F12 containing 3% dextran and either 1 mg/ml human HDL_3 protein (experiment 1, Tables 1 and 2) or 1% BSA (experiment 2, Tables 1 and 2) for 12 h. They were homogenized and then fractionated or the caveolae were isolated, and analyzed as described below.

CHO cells used for plasma membrane blebbing experiments were grown on Ham's F12 bicarb with 7.5% fetal bovine serum. When about 50% confluent, they were rinsed 3 times with 10 ml/plate of PBS with Mg^{2+} and Ca^{2+} and labeled with either [^{3}H]desmosterol (0.33 μCi/ml) or [^{14}C]cholesterol (0.1 μCi/ml) in Ham's F12 bicarb with 8 μg/ml of POPC, 5 μg/ml of cholesterol, 1 μm Triparanol, and 2 mg/ml of DLP for 48 h. After 48 h, cells were rinsed 3 times with PBS with Mg^{2+} and Ca^{2+}, 10 ml/dish, and then exposed to a 1-h rinse of Ham's F12 bicarb with 2 mg/ml of DLP at 37°C. Cells were then blebbed as described below.

Cell fractionation. To isolate plasma membranes for sterol analysis, cells were fractionated by the Gruber et al. modification of the Brunette-Till method (11), which is based on mixing and then separating aqueous solutions of polyethylene glycol (PEG) and dextran T-500. Plasma membranes are found at the interface and other organelles and membranes form a pellet. Cells were removed from plates after a brief incubation with PBS without Ca^{2+} and Mg^{2+} at 37°C using a rubber policeman. The cell suspension was transferred to a conical centrifuge tube and pelleted at 350 *g* for 5 min. Buffer was removed and cells were swelled using 30 mm $NaHCO_3$, pH 7, for 15 min at 20°C with gentle shaking. A drop of suspension was set aside on a microscope slide. All the subsequent steps were done on ice, and centrifuges and rotors were kept at 4°C. Cells were disrupted using a ball-bearing homogenizer, with a total bearing clearance of 41 micrometers, using 4–6 passes. A drop of homogenate was placed on a microscope slide and the pre- and post-homogenization suspensions were compared to estimate the amount of cell disruption. Care was taken not to break the cellular nuclei to avoid the release of DNA. Typical disruption of cells was between 70–80%. Homogenates were put in a conical tube and centrifuged at 835 *g* to pellet the nuclei and the undisrupted cells. The post-nuclear supernatant was transferred to a 50-ti tube (Beckman) and centrifuged at 12,000 *g* for 30 min. One ml of the supernatant was collected and stored on ice for subsequent analyses. The pellet was suspended in PEG top phase. The suspension was run through the ball-bearing homogenizer 4 times to disperse any membrane clumps and then mixed gently with an equal volume of dextran T-500 bottom phase. This preparation was centrifuged in a swinging bucket rotor for 30 min at 27,600 *g*. The solutions separated into two phases with PEG on the top, dextran T-500 on the bottom, plasma membranes at the interface, and intracellular membranes in the pellet. The PEG phase was discarded and the interface (IF1) was resuspended in 7 ml of 0.25 m sucrose, 2 mm EGTA, pH 7, and then pelleted by centrifuging at 125,000 *g* for 1 h. The resulting pellet was partitioned a second time between PEG and dextran and the resulting purified plasma membranes (IF2) were resuspended in sucrose/EGTA solution. Analytical

data are shown in Results for whole-cell extracts, post-nuclear supernatant (PNS), the purified plasma membranes (IF2), and the plasma membrane-depleted pellet of the first partitioning (pellet 1, PL1).

Protein and sterol analysis. Protein was measured by the Markwell et al. modification of the Lowry method (12) using BSA as the standard. Lipids were extracted from subcellular fractions and from incubation media using the method of Bligh and Dyer (13), dried under nitrogen, and saponified in 250 μl of 10% ethanolic potassium hydroxide for 1 h at 60°C. Non-saponifiable lipids were dissolved in acetonitrile–isopropanol 1:1. Comparative whole-cell lipids were extracted from the monolayers using HPLC-grade isopropanol and saponified. Cholesteryl methyl ether in toluene was added as an internal standard.

HPLC. Lipids were analyzed using HPLC as previously described (4). Briefly, samples were run on a reverse-phase C18 column (Supelcosil), 25 cm × 4.6 mm (id), 5 μm phase with a particle pore size of 120 angstroms. The mobile phase consisted of acetonitrile–isopropanol 3:1 and was run at 1.5 ml/min. Radioactivity was measured with a Radiomatic Flo-One\Beta series A-200 detector with the mobile liquid scintillant phase (Ultima Flo-M, Packard) at 1 ml/min. Retention times and the delay between UV and scintillation detection were monitored daily with known standards. Data were analyzed using Radiomatic's 500TR Series Window's compatible software (Packard). Peaks correspond to sterol standards as follows: P100, cholesterol; P70-74, desmosterol; P80-82, 7-dehydrocholesterol; P90 is unidentified (see Fig. 1a). The numbers in these designations indicate the retention time relative to cholesterol. [^{3}H]cholesteryl methyl ether was used as an internal standard for some analyses (P169-175).

Marker enzyme assays. Alkaline phosphodiesterase (plasma membrane marker) was monitored by the method of Beaufay et al. (14), using thymidine 5′-monophosphate nitrophenyl ester as the chromogenic substrate. N-acetyl-β-glucosaminidase, (lysosomal membrane marker), was measured by the method of Harrison, Smith, and Goodman (15), using *p*-nitrophenyl N-acetyl-β-d-glucosaminide as the chromogenic substrate. Changes in absorbance were monitored with a Beckman DU 40 spectrophotometer.

[^{3}H]cholesteryl methyl ether synthesis. [^{3}H]CME was synthesized from [^{3}H]cholesterol by the method of Dusza, Joseph, and Bernstein (16). Briefly, 100 μCi of [^{3}H]cholesterol in ethanol was placed in a disposable glass test tube, dried under nitrogen, then dissolved in 100 μl of trimethylorthoformate using a magnetic flea to solubilize. An aliquot (5.9 μl) of 60% perchloric acid was added using a Wiretrol with a Teflon plunger (Drummond). The sample was mixed gently for 15 min as a white precipitate formed. Two ml of aqueous saturated $NaHCO_3$ was added, and the tube was loosely capped and allowed to sit for 1 h with occasional gentle mixing. Three ml of methylene chloride was added and thoroughly mixed with the cap off to form two phases. After recapping, the sample was kept on a rocking platform for 1 h, to ensure the dissolution of the CME in the methylene chloride phase, and then centrifuged at 835 *g* for 15 min. The bottom methylene chloride phase was removed and placed in a clean tube. The aqueous phase was re-extracted with 3 ml of methylene chloride and centrifuged at 835 *g* for 15 min and this was combined with the first methylene chloride extract. The methylene chloride extract was washed with 3 ml of deionized water, which was aspirated and discarded after centrifugation at 835 *g* for 5 min. The sample was dried under nitrogen and dissolved in HPLC-grade acetonitrile–isopropanol 1:1. One percent aliquots were counted to determine initial recovery. Ninety percent was injected into the HPLC for purification (see Methods above). Fractions were collected at one minute intervals. [^{3}H]CME eluted at 19 min. Fractions from 17–20 min were combined and 1% was analyzed using HPLC to verify purity. One percent of each fraction was counted to determine recovery. Total yield after purification was 98%.

Preparative biosynthesis of [^{3}H]desmosterol. Mouse L-cells were grown in 100-mm plates for 48 h in bicarbonate-buffered MEM with 10% FBS. J774 macrophages were grown in bicarbonate-buffered RPMI with 10% FBS for 48 h. Plates were rinsed 3 times with PBS, and medium containing DLP (2 mg/ml) was applied for an additional 48 hours. Plates were washed again with PBS and medium containing 25 μCi [^{3}H]acetate/ml and 1% (w/v) BSA, buffered with HEPES instead of bicarbonate, was added. Bicarbonate was omitted to minimize the incorporation of [^{3}H]acetate into fatty acids. After 24 h, the medium was removed, plates were rinsed twice with PBS, and cellular lipids were extracted using HPLC-grade isopropanol. [^{3}H]CME (0.25 μCi) was added to each plate as an internal standard. Samples were saponified as described above. Approximately 90% of the lipid from each plate was purified using HPLC, with one fraction collected between 0–6 min, and then one fraction per min for the next 20 min. [^{3}H]desmosterol eluted at 8.4 min. Fractions from 7.5–8.75 minutes were combined and 1% was analyzed by HPLC to verify purity. [^{3}H]CME was used to calculate loss during manipulation of samples. Two 100-mm plates of the two cell types (4 plates total) produced 14 μCi of [^{3}H]desmosterol with a specific activity of about 2.0×10^5 DPM/μg.

Caveolae isolation and western blotting. Caveolae were isolated using the method of Sargiacomo et al. (17). Briefly, cell homogenate in 2 ml of a 45% sucrose solution was placed in the bottom of a centrifuge tube. Ten ml of a sucrose step gradient, from 30–7.5% sucrose was laid by hand over the sample and centrifuged at 143,000 *g* for 18 h at 4°C. The sucrose gradient was fractionated into approximately 18 650-μl fractions and the density of each was estimated from its refractive index. To analyze for caveolin-1, proteins were precipitated from 325 μl of each fraction using 10% trichloroacetic acid and solubilized in gel-electrophoresis running buffer of 125 mm Tris-HCl, pH 6.8, 5% glycerol, 2% sodium dodecyl sulfate, 0.003% bromophenol blue, and 10% β-mercaptoethanol. Samples were heated at 100°C for 10 min in a heating block. Denaturing polyacrylamide gel electrophoresis was performed following the method of Laemmli (18) using high tensile acrylamide. Proteins were run on a continuous 12% gel with rainbow molecular weight standards and alkaline phosphatase-conjugated molecular weight standards. Gels were blotted onto a polyvinylidene difluoride (PVDF) membrane using a semi-dry blotting transfer system with a graphite electroblotter at 0.725 amperes for 30 min (Millipore). Blots were treated according to the caveolin antibody manufacturer's instructions (Transduction Laboratories, Protocol #4, Western Blotting with Monoclonal Antibodies).

Plasma-membrane blebbing. CHO cells were labeled and rinsed as described above, and then plasma membrane "blebs" were obtained using the method of Bellini et al. (19) with two slight modifications. *1*) The formaldehyde/dithiothreitol solution was left on the cells overnight to maximize bleb formation, and *2*) both groups of cells were labeled in the presence of Triparanol to prevent the conversion of [^{3}H]desmosterol to [^{3}H]cholesterol. Blebs were resuspended and mixed with human HDL_3 at a concentration of 1 mg HDL_3 protein/ml and approximately 18 μg of plasma-membrane cholesterol/ml. Blebs and HDL_3 were incubated at 37°C in a rotary water bath and at various time points aliquots were removed and passed through 0.45-μm filters. Blebs were retained by the filter, while HDL_3 eluted. The radioactivity in the HDL_3 was measured by liquid scintillation counting, and the percent transfer of radioactive sterol from blebs to HDL_3 was calculated.

LUV vesicle preparation and separation. Stock solutions of lipids in chloroform were added to 16 mm × 100 mm disposable glass

tubes at the appropriate mole ratios, and first dried under nitrogen and then under vacuum overnight to remove any residual solvent. Multilamellar vesicles were generated after the addition of 20 mm HEPES and 10 mm NaCl, pH 8.0, followed by vortexing to disperse the dry lipid film. Donor and acceptor large unilamellar vesicles (LUVs) were formed after forcing multilamellar vesicles through two stacked 100-nm pore size polycarbonate filters housed in an extruder (Lipex Biomembranes) using nitrogen pressures of 300–400 psi as previously described (20). Donor vesicles were composed of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC)/1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG)/sterol (either cholesterol or desmosterol) at molar ratios of 75:15:10. In some cases, donor vesicles contained both cholesterol and desmosterol and were composed of POPC–POPG–cholesterol–desmosterol at molar ratios of 65:15:10:10. Donor vesicles containing sphingomyelin were composed of sphingomyelin–POPC–POPG–sterol at molar ratios of 35:40:15:10 and prepared as described above except that extrusion was performed at 54°C. Acceptor LUV consisted of POPC with a tracer quantity of [^{3}H]CHE (1 μCi/ml LUV) to monitor recovery. At pH 8.0, POPG incorporated into donor vesicles imparted an overall negative charge, which aided in their separation from acceptor vesicles. Incubations were carried out in a stationary water bath at 37°C in 20 mm HEPES and 10 mM NaCl, pH 8, with acceptors at 6 mg and donors at 0.75 mg phospholipid/ml (donors:acceptors, 1:8). LUV donors were separated from LUV acceptors with minor modifications to the method of Rodrigueza et al. (20), using DEAE Sephadex A-25. Start to finish, the separations were completed in 4–5 min. DEAE Sephadex was hydrated 1:1 (v/v) with buffer. Columns were prepared immediately before separating the sample. The slurry was packed into 1-ml tuberculin syringes, which were plugged with glass wool and centrifuged for 1 min at 55 *g* at 4°C. Additional packings with the same centrifugation conditions were carried out until the syringe was packed to the 1 ml mark and the buffer was removed from the column by a final centrifugation for 2 min at 55 *g*. At each time point, 100–200 μl of sample was applied to the top of the column and the syringes were centrifuged for 1 min at 835 *g*. To maximize acceptor recovery, two 200-μl rinses of buffer were applied to each column and centrifuged for an additional 2 min at 835 *g*. Acceptor recoveries were between 60–70%, based on [^{3}H]CHE radioactivity in the column eluant. One to 5% of eluant was counted using liquid scintillation counting, and the remainder was analyzed for sterol masses using gas–liquid chromatography, following the procedure of Ishikawa et al. (21), as modified by Klansek et al. (22). Sterol transfer was calculated as:

$$\text{percent sterol transfer} = \frac{\left(\frac{\mu\text{g sterol recovered in acceptor}}{\text{fractional recovery of acceptor}}\right)}{\mu\text{g sterol in starting donor}} \times 100$$

In preliminary experiments, [^{3}H]CHE was incorporated into the donors to confirm that there was no leakage of these vesicles from the column. Typically, about 0.2% of labeled donors came through the column, and this did not change over the time course of the incubations (data not shown). These data indicate that [^{3}H]CHE is essentially nonexchangable.

Sterol transfer from LUV to HDL$_3$ and LDL. HDL$_3$ and LDL were isolated from fresh human plasma by the method of Hatch and Lees (23), and dialyzed exhaustively using Spectra/Por molecular porous dialysis membrane with a molecular mass cutoff of 3,500 Daltons, against 0.15 m NaCl. Donor LUV were composed of POPC/[^{14}C]cholesterol or [^{3}H]desmosterol, with a molar ratio of 90:10, or both sterols together, POPC–cholesterol–desmosterol, molar ratio of 80:10:10, in a buffer of 10 mm NaH_2PO_4 and 150 mm NaCl, pH 7.4. Sterol specific activities for [^{14}C]cholesterol and [^{3}H]desmosterol in donor LUV were 6,700 and 8,800 CPM/μg, respectively. Concentrations of incubation components were as follows: donor LUV, 1.4 mg phospholipid/ml; HDL$_3$, 1.4 mg protein/ml; LDL 1 mg protein/ml. The incubation temperature was 37°C. To separate donor LUV from acceptor lipoproteins, duplicate 5-μl samples were loaded into lanes of separate halves of a Beckman Paragon 0.5% agarose Lipogel. Samples were allowed to soak into the gel matrix for 20 min before electrophoresis. Gels were run at 100 volts for 45 min and then were cut longitudinally in half, so that, of the duplicate lanes, one was fixed and stained to determine the migration of the lipoproteins and LUV, and the other lane was cut into 7-mm sections, which underwent scintillation counting. Percent transfer of sterol to HDL$_3$ or LDL was calculated from the counts recovered with the appropriate migration (α or β), divided by the total counts recovered from the lane. Total recovery of radioactivity was usually 60%, calculated from total-lane recovery, divided by the counts in 5 μl of the incubation system.

Statistical analysis. Most data are reported as the mean ± one standard deviation of triplicate determinations. Where indicated, some values are means ± one-half the range of duplicate values. For triplicate determinations, data were analyzed using Student's *t*-test. Comparisons were paired when feasible (e.g., when desmosterol and cholesterol transfer from the same LUV were measured). Otherwise, the unpaired *t*-test was used.

RESULTS

Sterols in bulk plasma membrane

The enhanced release of newly synthesized desmosterol in comparison to newly synthesized cholesterol could be due either to more efficient delivery to the plasma membrane or to more facile desorption from the plasma membrane to extracellular acceptors. To distinguish between these possibilities, we examined the newly synthesized sterols contained in the plasma membrane of CHO-K1 cells in comparison to those in whole cells. Elevation of the ratio of newly synthesized desmosterol-to-cholesterol ratio in the plasma membrane would suggest more efficient delivery of desmosterol to the plasma membrane. We also measured the release of the newly synthesized sterols into medium that contained an extracellular cholesterol acceptor; in these experiments, we used human HDL$_3$. If the ratio of the newly synthesized desmosterol to cholesterol is elevated in HDL$_3$ compared to the membrane lipids, it would suggest that there is a preferential desorption of desmosterol from the plasma membrane.

Fractions were obtained consisting of either purified plasma membranes (IF2 from two-phase partitioning procedure) or PL1 (consisting of plasma membrane-depleted cellular particulates) using the Gruber modification (11) of the Brunette-Till method. These methods are reported to generate plasma membranes with only 3.7% of contaminating membrane from sources such as mitochondria, lysosomes, endoplasmic reticulum, and Golgi apparatus (11). With our fractions, marker enzyme assays for APDase and NABGase (plasma membrane and lysosomes, respectively) showed that the yield of plasma membrane in IF2 was rather low (8.5–21% of cell homogenate), but this fraction exhibited a 13- to 21-fold enrichment of APDase

TABLE 1. Membrane and organellar marker assays

Enzyme, Fraction	μg Protein / 10^7 Cells	ΔA400 / (h * 10^7 Cells)	% Yield	ΔA400 / (h * mg Protein)	Fold Enrichment
Experiment 1[a]					
APDase					
PNSPNS	637 ± 4	2.01 ± 0.06	100	3.1 ± 0.3	1
Plasma membrane	≤4[c]	0.17 ± 0.01	8.5	≥42	≥13
Other membranes	71 ± 5	0.48 ± 0.01	24	6.8 ± 0.6	2.2
NABGase					
PNS		2.76 ± 0.03	100	4.3 ± 0.3	1
Plasma membrane		0.03 ± 0.01	1.0	≥7.3	≥1.7
Other membranes		1.1 ± 0.1	39	15 ± 2	3.5
Experiment 2[b]					
APDase					
PNS	137 ± 1	0.48 ± 0.06	100	3.5 ± 0.5	1
Plasma membrane	1.44 ± 0.04	0.102 ± 0.001	21	73 ± 5	21
Other membranes	10 ± 0.4	0.091 ± 0.001	19	9.3 ± 0.5	2.6
NABGase					
PNS		1.0 ± 0.1	100	7.4 ± 0.8	1
Plasma membrane		0.04 ± 0.01	4.3	30 ± 2	4.0
Other membranes		0.11 ± 0.01	11	11 ± 1	1.4

Plasma membrane(IF2) and other membranes (PL1) were from the two-phase partitioning procedure (see Materials and Methods). PNS is the post-nuclear supernatant. Percent yield was calculated based on the change in absorbance normalized to 10 million cells over one h, with that in the PNS representing 100% of enzyme activity. Fold enrichment was calculated based on specific activities, with PNS representing 1.

[a]Cells were incubated for 12 hours with HDL_3 (1 mg protein / ml) and [^{3}H]acetate(25 μCi / ml) immediately prior to homogenization.

[b]Cells were incubated for 12 hours with 1% BSA (no HDL_3) and [^{3}H]acetate (25 μCi / ml) immediately prior to homogenization.

[c]Protein value was within the indicated measurement error for this experiment.

specific activity compared to a 2- to 3-fold enrichment in PL1 (**Table 1**). NABGase fold enrichment in PL1 was between 1 and 3 compared to a 2- to 4-fold enrichment in the plasma membrane fraction. This suggests that the IF2 fraction, although containing some other particulate material, was greatly enriched in plasma membranes compared to both the PNS and PL1 from the two-phase partitioning procedure. Our fold enrichment of APDase in plasma membrane compares well with examples from the literature, where enrichments in the range of 7- to 34-fold are reported (24, 25).

Next we measured the sterols contained in the membranes and compared them to those in whole cells and medium. HPLC analysis of nonsaponifiable lipids showed peaks corresponding to desmosterol (P70), 7-dehydrocholesterol (P80), cholesterol (P100) and an unidentified peak 90 (all retention times relative to cholesterol) (**Fig. 1**). In general the HPLC profiles for whole cells and plasma membrane were similar, whereas there was a large relative increase in the desmosterol peak in HDL_3. **Table 2** lists ratios of desmosterol to cholesterol in plasma membranes in three separate experiments. In two cases, the results showed similar ratios of desmosterol to cholesterol in the whole cells and in the isolated plasma membrane. In one experiment (#4 in Table 2), the ratio in plasma membranes is statistically different from whole cells, but it was decreased, not increased, as in the HDL_3 samples. HPLC profiles of plasma membrane sterols were similar when cells were incubated with an extracellular sterol acceptor (HDL_3) and without an acceptor (BSA), (sterol ratios in Table 2, experiments 1, 2, and 4). However, desmosterol levels relative to cholesterol were raised approximately 4-fold in the medium containing HDL_3, compared to isolated plasma membranes. In each experiment, the ratio of desmoserol to cholesterol in the HDL_3 is significantly higher ($P < 0.05$) than the ratio in whole cells and in the plasma membranes. This suggests that after 12 h, both cholesterol and desmosterol have reached the plasma membrane to the same extent, but that desmosterol dissociates from the cell surface with a greater efficiency than cholesterol.

Sterols in caveolae. We wished to determine whether the enhanced release of desmosterol might be due to an enhanced delivery of this sterol to caveolae. To investigate the sterol composition of caveolae, we labeled cells with [^{3}H]acetate for 12 h and isolated the caveolae as Triton X-100-insoluble complexes which were then fractionated from a cellular homogenate on a linear gradient of 7.5–42.5% sucrose. Intact caveolae are known to have a density corresponding to 10–20% sucrose (17). Western blot analysis (**Fig. 2**) indicated an isolation of the majority of cellular caveolin-1 at this density (fractions 8–12). Caveolin found at higher densities (fractions 14–18, 22–34% sucrose) probably was derived from intracellular caveolin protein associated with the endoplasmic reticulum and the Golgi apparatus (26). We analyzed the nonsaponifiable lipids from the whole cells and medium containing HDL_3, and compared these to the lipids contained in the caveolae (fractions 8–12), avoiding the higher density non-caveolar fractions. The HPLC sterol profiles were similar in caveolae and whole cells (**Fig. 3**) and the desmosterol-to-cholesterol ratio did not differ significantly in any of

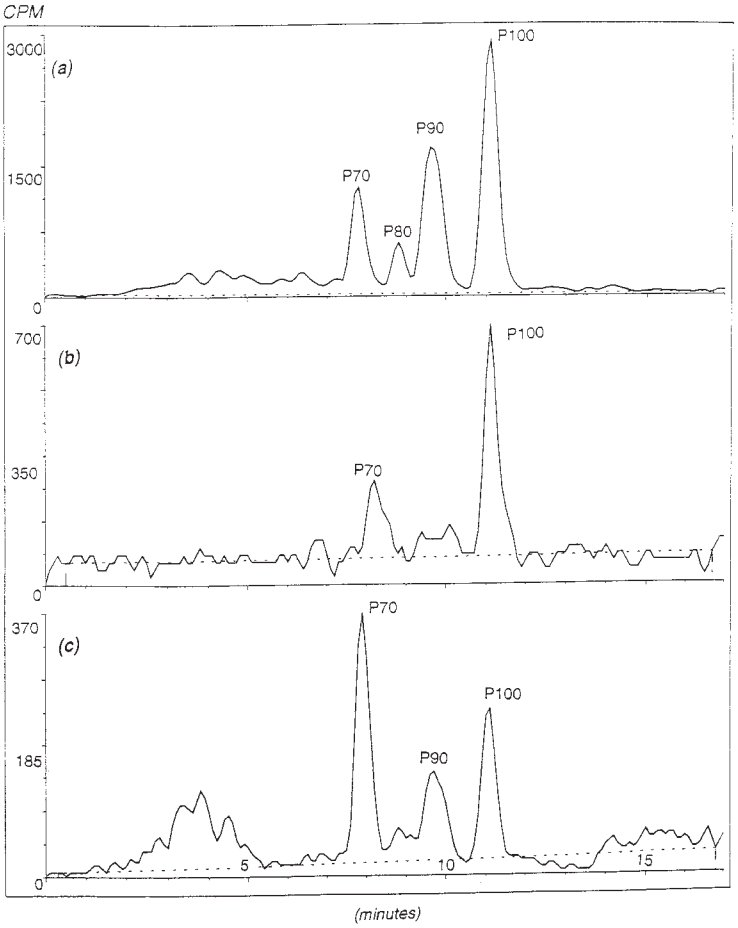

Fig. 1. Radioactive HPLC profiles of biosynthetic sterols in: (a) whole CHO-K1 cells; (b) plasma membranes (IF2), and (c) HDL_3 incubation medium. All counts are from lipids that were synthesized during a 12-h exposure to [^{3}H]acetate. Cholesterol corresponds to peak 100 and desmosterol corresponds to peak 70. Data are from experiment number 2 (see Tables 1 and 2).

the experiments between caveolae and whole cells, whereas the ratio in HDL_3 was significantly elevated in comparison to caveolae (Table 2). These results suggest that both sterols have nearly equal access to plasma membrane caveolae, and that selective deposition of desmosterol in caveolae is unlikely to account for its enhanced efflux.

Sterol transfer from isolated plasma membrane to HDL_3. To confirm the ability of desmosterol to desorb more readily than

TABLE 2. Ratios of radioactive desmosterol to cholesterol in whole cells, isolated plasma membranes (IF2) and caveolae, and released to medium containing HDL_3

	Normalized Ratios of [^{3}H]Desmosterol to [^{3}H]Cholesterol[a]			
Experiment	Des/Chol in Whole Cells	Des/Chol in Plasma Membranes	Des/Chol in Caveolae	Des/Chol Released to HDL_3
1[b]	1 ± 0.3	0.73 ± 0.4	ND	2.8 ± 0.4
2[c]	1 ± 0.06	1.1[f]	0.70 ± 0.18	4.7 ± 1.1
3[d]	1 ± 0.15	ND	0.95 ± 0.79	ND
4[e]	1 ± 0.1	0.54 ± 0.02	0.79 ± 0.26	2.4 ± 0.1

[a] In each experiment, the whole-cell ratio was set at 1. All other values for that experiment are given with respect to that value; ND, not determined.

[b] Cells were incubated for 12 hours with HDL_3 (1 mg protein/ml) and [^{3}H]acetate (25 μCi/ml) immediately prior to homogenization. The measured [^{3}H]desmosterol/[^{3}H]cholesterol ratio in whole cells was 0.7 ± 0.2 (cpm/cpm).

[c] Cells were incubated for 12 h with 1% BSA (no HDL_3) and [^{3}H]acetate (25 μCi/ml) immediately prior to homogenization. Two additional plates (not subjected to fractionation) were incubated with HDL_3 (1 mg protein/ml) to monitor sterol efflux. (Same as experiment 2 in Table 1). The measured [^{3}H]desmosterol/[^{3}H]cholesterol ratio in whole cells was 0.33 ± 0.02 (cpm/cpm).

[d] Cells were incubated for 12 h with 50 mg/ml of BSA and [^{3}H]acetate (25 μCi/ml) immediately prior to homogenization. The measured [^{3}H]desmosterol/[^{3}H]cholesterol ratio in whole cells was 0.62 ± 0.09 (cpm/cpm).

[e] Cells were incubated for for 12 h with HDL_3 (1 mg protein/ml) and [^{3}H]acetate (25 μCi/ml) immediately prior to homogenization. The measured [^{3}H]desmosterol/[^{3}H]cholesterol ratio in whole cells was 0.4 ± 0.04 (cpm/cpm).

[f] Recovery of radioactivity was low, so triplicate samples were combined.

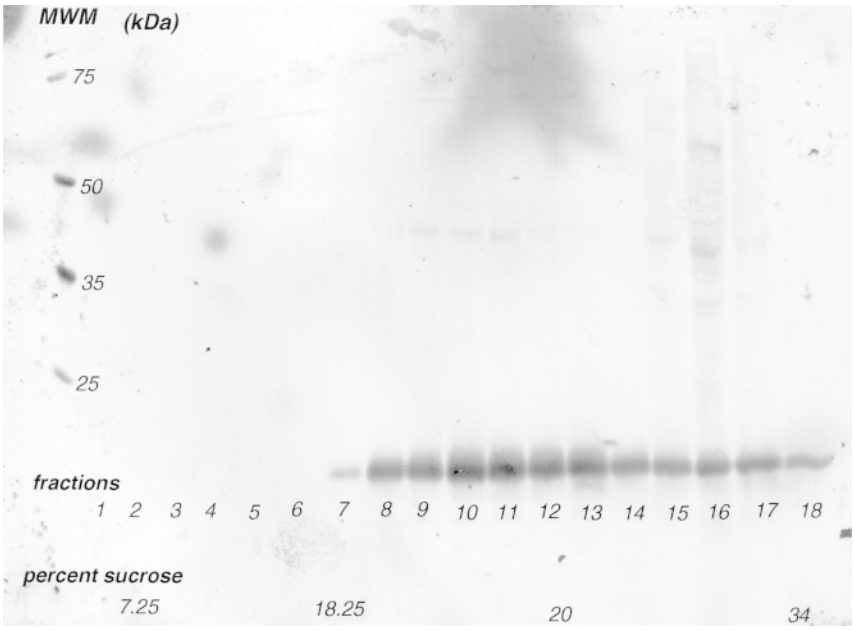

Fig. 2. Western blot of caveolin-1 protein in subcellular fractions of CHO-K1 cells. Molecular weight markers (lane 0) confirmed that the caveolin immunoreactivity migrated to the region of approximately 22 kDa, the known molecular mass of this protein. Densitometric analysis of the 22-kDa band intensities indicated a bimodal distribution of caveolin, with peaks at fractions 11 (caveolae) and 16 (detergent-soluble caveolin). For analysis of caveolar sterols (Fig. 3), fractions 8–12 (18.2–20% sucrose) were analyzed. Data are from experiment number 3 (see Table 2).

cholesterol from the plasma membrane, we labeled CHO cells with [^{3}H]desmosterol or [^{14}C]cholesterol, isolated plasma membrane vesicles from these cells, and then measured the transfer of labeled sterols from these vesicles to HDL_3 (19, see also Methods). The labeling incubation included triparanol to inhibit any conversion of desmosterol to cholesterol. Over a 4-h time course, the transfer of desmosterol was 2.7 times more rapid than that of cholesterol (**Fig. 4**). Thus the difference in the transfer of the two sterols persists in the cellular plasma membrane without the participation of intracellular sterol transport.

Sterol transfer from LUV donors. To determine whether the enhanced efflux of desmosterol could be explained by a difference in the association of the sterols with the phospholipid bilayer, we examined cholesterol and desmosterol transfer from LUV to LUV. As described in Materials and Methods, donor vesicles were negatively charged due to the incorporation of POPG, whereas acceptor vesicles were neutral. Incubations were set up with an 8-fold excess of acceptor LUV. Recovery of acceptors was accomplished by DEAE-Sephadex chromatography. In the standard procedure, acceptor recovery was quantified based on recovery of [^{3}H]CHE, a non-exchangeable tracer incorporated into the uncharged acceptor vesicles (27).

The time course of cholesterol and desmosterol transfer in the LUV system from 0 to 8 h is shown in **Fig. 5**. We found that the initial rate of transfer could be estimated by the 2-h time point, and that with both sterols, the transfer had approached equilibrium by 8 h. As observed with intact cells, desmosterol transferred from LUV two to three times more rapidly than cholesterol . Repeated experiments showed that 20–40% of cholesterol transferred at 2 h, while 70–80% of desmosterol transferred (**Table 3**). There were no changes in the rates of release when the sterols were combined in the same vesicles, suggesting that the enhancement of desmosterol release is not dependent on the presence of cholesterol in the membrane. We found that the rates of release of both sterols (expressed as percent transfer) were not altered in any consistent pattern by changes in the concentration of sterol in the LUV (3–40 mole %) (**Fig. 6**).

To examine the influence of sphingomyelin on the selectivity of sterol transfer, we made LUV that contained 35 mole % sphingomyelin, a level close to that found in the outer leaflet of the plasma membrane (28). We made donor vesicles with either egg sphingomyelin or BBSM, to determine whether the different acyl chain compositions could alter the transfer rate of either sterol. Both types of sphingomyelin reduced the transfer of both sterols by approximately 70%, but had no major effect on the relative amounts of transfer of cholesterol and desmosterol (**Table 4**). Thus, the greater transfer rate of desmosterol was maintained when sphingomeylin was incorporated into donor LUV. There was no significant difference between the effects of the two types of sphingomyelin.

To determine whether the enhanced release of desmosterol would persist in the presence of human HDL_3 and LDL as the acceptor, LUV that contained either labeled cholesterol or demosterol, or both of the sterols together, were incubated with either HDL_3 or LDL and then separated using agarose gel electrophoresis. We saw the same enhanced release of desmosterol to both HDL_3 and LDL

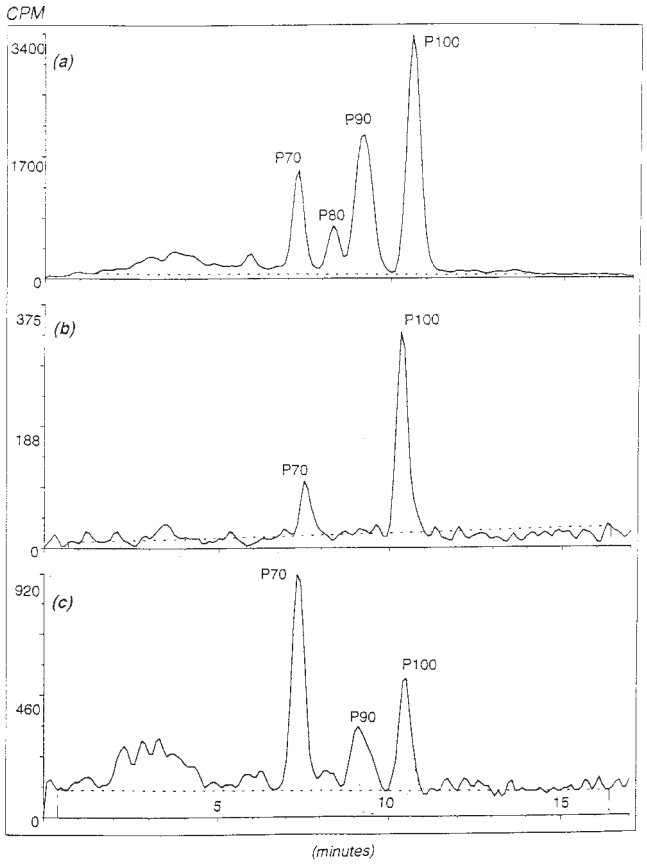

Fig. 3. Caveolar sterol composition. Radioactive HPLC profiles of sterols synthesized in CHO-K1 cells from [^{3}H]acetate during a 12-h incubation. Lipids were extracted from: (a) whole cells, (b) caveolae (fractions 8–12, corresponding to the blot in Fig. 2), and (c) HDL_3-containing medium. Data are from experiment number 3 (see Table 2).

with an initial rate of transfer two to three times that of cholesterol (**Table 5** and **Table 6**). No difference was seen between rates of transfer when sterols were in separate or in the same donor vesicles. Some transfer was apparent at the 0-h time point, but this probably occurred while loading the sample into the gel, which required approximately 20 min to accomplish.

DISCUSSION

We have shown that in CHO-K1 cells desmosterol is released 2 to 3 times more efficiently than cholesterol. The delivery of this intermediate to the plasma membrane does not appear to be more efficient than that of cholesterol, and the enhanced release persists in plasma membrane blebs, which lack intracellular transport machinery. It does not appear to be due to the preferential localization of desmosterol in the plasma membrane caveolae. Moreover, the enhanced release of desmosterol persists in the LUV's simple bilayer membranes that are devoid of protein. Therefore, the differential efflux of cholesterol and desmosterol from cells appears to be due to the manner in which these sterols associate with plasma membrane phospholipids and not to differential delivery to the cell surface or to sterol-specific mediation of efflux by plasma membrane proteins.

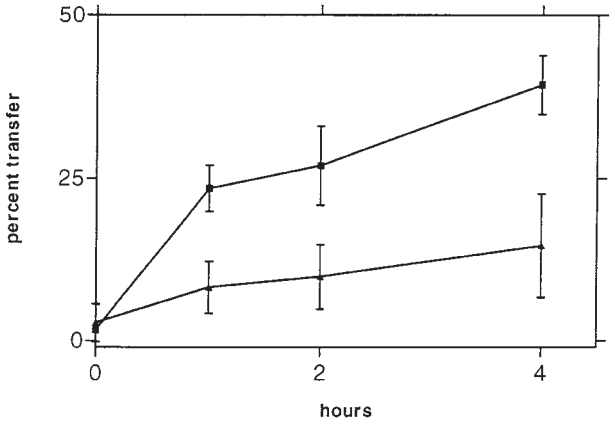

Fig. 4. Transfer of cholesterol (▲) and desmosterol (■) from CHO plasma membrane blebs. Right-side-out blebs were made from cells labeled with [^{3}H]desmosterol or [^{14}C]cholesterol and were then incubated with HDL_3 at 1 mg protein/ml. Percent transfer was measured as radioactivity associated with HDL_3 versus the starting bleb radioactivity. Background was measured by incubating blebs with buffer and filtering at each time point. This number was subtracted from each time point, and the standard deviations were added. This background averaged 21 ± 1% for cholesterol and 16 ± 3% for desmosterol and did not vary significantly between time points. Desmosterol transfer was significantly different from cholesterol ($P \leq 0.05$) at each time point.

We and others have reported on the accumulation of biosynthetic sterol intermediates in cells (3–5, 29), but the significance of this accumulation and fate of the intermediates are incompletely defined. It is confirmed that intermediates such as desmosterol, zymosterol and lanosterol

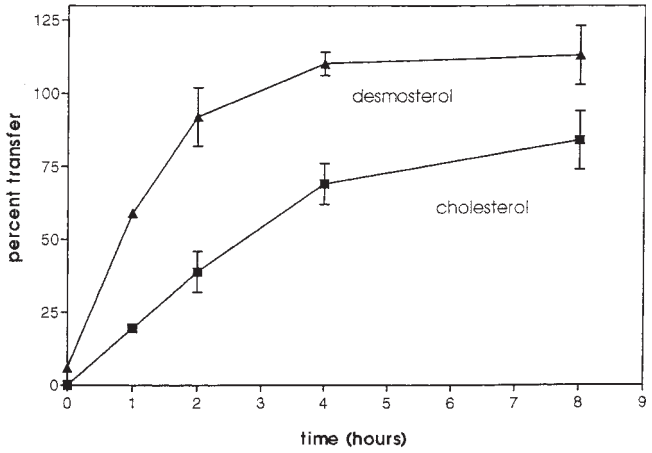

Fig. 5. Cholesterol and desmosterol transfer from LUV to LUV over 8 h. Donor LUV contained both desmosterol and cholesterol. Nonsaponifiable lipids were extracted from the acceptors and compared to beginning donor concentrations to calculate percent transfer. Acceptor recovery was monitored using [^{3}H]CHE. LUV concentrations were 0.75 mg donor phospholipid/ml and 6 mg acceptor phospholipid/ml (1:8).

TABLE 3. LUV-to-LUV transfer of cholesterol and desmosterol

Donor Vesicle, Experiment	% Cholesterol Transfer/2 h	% Desmosterol Transfer/2 h
Cholesterol and desmosterol in separate incubations		
Exp. 2	44 ± 6	
Exp. 3	21 ± 2	77 ± 9[a]
Exp. 4	29 ± 2	79 ± 21[a]
Cholesterol and desmosterol in the same donor vesicle		
Exp. 1	39 ± 8	92 ± 16[a]
Exp. 4	22 ± 5[b]	88 ± 6[a,b]

Each incubation contained donor vesicles with 10 mole % of a given sterol. In some cases the donor contained a single type of sterol. In others, the two sterols were combined in the same donor vesicle. LUV concentrations were as in Fig. 4.

[a]Significantly different ($P < 0.05$) from the corresponding cholesterol transfer value in the same row.

[b]Not significantly different from the corresponding same-sterol value in the single-sterol incubations from the same experiment.

TABLE 4. Effect of sphingomeylin in donor vesicles on LUV-to-LUV sterol transfer

Donor Vesicle, Experiment	% Cholesterol Transfer/2 h	% Desmosterol Transfer/2 h	Desmosterol/Cholesterol
No sphingomyelin (POPC and POPG)			
Exp. 1	21 ± 2	64 ± 4[a]	3
Exp. 2	30 ± 5	91 ± 16[a]	3
35 Mole % bovine brain sphingomyelin			
Exp. 1	6 ± 1[b]	24 ± 1[a,b]	4
Exp. 2	8 ± 2[b]	32 ± 3[a,b]	4
35 Mole % egg sphingomyelin			
Exp. 2	4 ± 1[b]	20 ± 9[a,b]	5

Each incubation contained a single type of sterol. LUV concentrations were as in Fig. 4.

[a]Significantly different ($P < 0.05$) from the cholesterol transfer value in the same row.

[b]Significantly different ($P < 0.05$) from the same-sterol transfer value obtained without donor sphingomyelin in the same experiment.

are transported to the plasma membrane of cells (3). Metherall, Waugh, and Li (29, 30) have shown in a variety of cells that this accumulation can be enhanced by treatment of cells with progesterone, an effect attributed to the ability of this steroid to inhibit one or more members of the MDR protein family, preventing return of intermediates in the plasma membrane to the endoplasmic reticulum for conversion to cholesterol. Lange, Echevarria, and Steck (5) have reported that newly synthesized zymosterol is transported to the plasma membrane in fibroblasts more efficiently than newly synthesized cholesterol, with half times of 9 and 18 min, respectively. Whereas Baum et al. (31) have reported that in McA-RH7777 cells the rate of transport of newly synthesized desmosterol to the plasma membrane is roughly equal to that of cholesterol, with half times of approximately 32 min for cholesterol and 42 min for desmosterol. In both fibroblasts (5) and McA-RH7777 cells (31) equal fractions of cholesterol and the relevant sterol intermediate had accumulated in the plasma membrane after an incubation time of 2 h. Similarly, after a 12-h labeling period in CHO-K1 cells, we saw little difference in the desmosterol-to-cholesterol ratios of whole cells

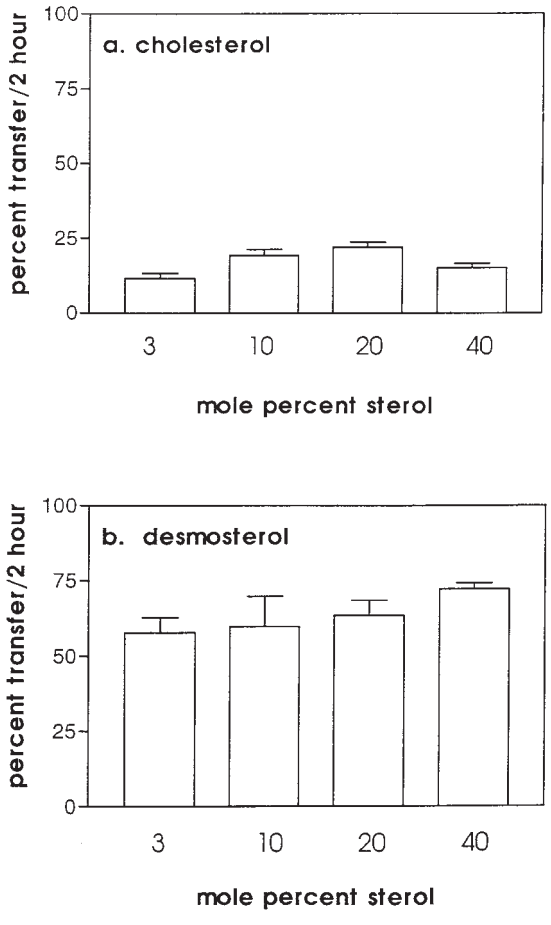

Fig. 6. Transfer of sterols from donor LUV containing different concentrations of sterols. Donor vesicles were made with 3, 10, 20, and 40 mole percent of either cholesterol or desmosterol, with compensatory decreases in the mole % of donor PC. Other conditions were as in Fig. 4. (a) Cholesterol transfer; (b) desmosterol transfer.

TABLE 5. Cholesterol and desmosterol transfer from LUV to human HDL_3

Experiment, Hour	% Cholesterol Transfer	% Desmosterol Transfer	% Cholesterol Transfer in the Presence of Desmosterol	% Desmosterol Transfer in the Presence of Cholesterol
Exp. 1				
0	5.6 ± 0.4	16 ± 1		
2	10 ± 2	32 ± 2		
8	18 ± 3	35 ± 1		
Exp. 2				
0	4.18 ± 0.02	13 ± 2	5 ± 1	13 ± 4
2	15 ± 2	40 ± 3	18 ± 2	34 ± 2
Exp. 3				
0	3.9 ± 0.3	2 ± 1		
2	13 ± 0	30 ± 3		
8	17 ± 1	34 ± 2		

Sterols were either in separate donor LUV or combined in the same donor LUV. LUV concentration was 1.4 mg phospholipid/ml and HDL_3 concentration was 1.4 mg protein/ml. Cholesterol vs. desmosterol, 2 h, $0.01 \geq P \geq 0.001$ for all experiments. Cholesterol vs. desmosterol, 8 h, $0.01 \geq P \geq 0.001$ for both available experiments.

TABLE 6. Cholesterol and desmosterol transfer from LUV to human LDL

Experiment, Hour	% Cholesterol Transfer to LDL	% Desmosterol Transfer to LDL
Exp. 1		
0	4.6 ± 1	9 ± 2
2	16 ± 1	44 ± 1
8	24 ± 1	53 ± 1
Exp. 2		
0	3 ± 0.4	12 ± 1
2	11 ± 1	31 ± 1

Sterols were in separate donor LUV. LUV concentration was 1.4 mg phospholipid/ml and LDL concentration was 1 mg protein/ml. Cholesterol vs. desmosterol, 2 h, $P = 0.0001$, using measurements from experiments 1 and 2. Cholesterol vs. desmosterol, 8 h, $P = 0.0001$, using measurements from experiment 1.

and isolated plasma membranes, implying that equal fractions of the two sterols had been delivered to the plasma membrane. Regarding any impact on efflux of biosynthetic sterols, half-times of less than an hour for intracellular transport would mean that this step is not rate-limiting for efflux, as desorption from the cell surface in the presence of plasma lipoproteins occurs with half-times on the order of hours (32). Thus differences in the short half-times of intracellular transport would not be expected to produce large differences in efflux. It might be suggested that the rate of transport of desmosterol to the cell surface is faster, but that over time the concentrations of the two sterols in the membrane are similar because so much desmosterol is released to the extracellular HDL_3. However, we saw similar sterol profiles in plasma membranes from cells incubated without an extracellular sterol acceptor (BSA) and in plasma membranes from cells incubated with HDL_3.

The plasma membrane appears to contain cholesterol-rich and cholesterol-poor regions (33). Currently, researchers are focusing on cellular caveolae as a cholesterol-rich plasma membrane domain that could play a role in the efflux of sterols from cells (34, 35). Caveolae are flask-shaped invaginations on the cell's surface that contain a structural protein, caveolin, which has a unique ability to bind cholesterol. The exact function of caveolae is undetermined, but they are diminished in number on the surface of cells that are starved of cholesterol. Fielding and Fielding (35) have reported that caveolae mediate rapid efflux of cellular cholesterol to HDL and also are the region where cells sequester recently synthesized sterols, thus possibly playing a role in their efflux. Recently, Smart et al. (36) have reported that newly synthesized sterols are transported to the plasma membrane and delivered directly to the caveolae. From here they can diffuse laterally through the membrane. Our results indicate that the selective deposition of desmosterol into caveolae after 12 h of labeling does not account for the enhanced efflux of this sterol. This finding does not argue either for or against the importance of caveolae in cholesterol efflux, nor does it determine whether the desmosterol is initially delivered to this plasma membrane structure.

In contrast with the present results, Clejan and Bittman (37) have reported that desmosterol is released from the membranes of mycoplasma cells at a rate slower than cholesterol. Thus, it appears that mycoplasma and eukaryotic plasma membranes may differ in the selectivity of their sterol efflux. Possible explanations for this difference include: *1*) the phospholipid composition of the mycoplasma membrane, consisting entirely of phosphatidylglycerol (38), which is much different from the phospholipid composition of the mammalian cell plasma membrane or of the LUV used in the present studies, and *2*) the reported difference in the abilites of cholesterol and desmosterol to translocate (flip-flop) across the mycoplasma membrane, with exogenous desmosterol confined to the outer leaflet, and cholesterol distributed between the two membrane leaflets (37, 38). At present, it is not possible to conclude whether these or other features of the mycoplasma cell account for the discrepancy with the present results.

The more rapid release of desmosterol from LUV could be due to a lower strength of association of this sterol with the membrane phospholipids. However, physical chemical studies show no difference between cholesterol and desmosterol in their abilities to condense phospholipid monolayer films (39). Decreased condensation occurs in phospholipid membranes containing sterols that do not have planar ring systems (coprostanol), lack the isooctyl side chain (androstanol), have methylations at C_4 (lanosterol), have an α-oriented hydroxyl group (epicholesterol) or a carbonyl group at C_3 (40–42). This suggests that the desmosterol molecule associates with the bilayer phospholipids as strongly as cholesterol. Thus, its enhanced release may be due solely to its decreased hydrophobicity, which would reduce the activation energy required for the molecule to desorb into the aqueous surroundings.

Sphingomyelin is an important phospholipid constituent of the cellular plasma membrane, and due to its association with cholesterol, has been shown to decrease the transfer of cholesterol to extracellular acceptors. Because of its major role in the structure of the plasma membrane, it was important to characterize the effect of sphingomyelin on the transfer of cholesterol and desmosterol. The addition of sphingomyelin to donor LUV reduced the rates of transfer for both cholesterol and desmosterol, but the large relative enhancement of desmosterol release persisted. Thus, the presence of high concentrations of sphingomyelin in the outer leaflet of the plasma membrane probably reduces the efflux of sterols, but does not eliminate the enhanced efflux of desmosterol relative to cholesterol.

One of the more interesting facts about the sterol biosynthetic intermediates is that they are associated with and may cause pathology in humans and higher animals. In the Smith-Lemli-Opitz syndrome (SLOS), there is a deficiency of sterol-Δ-7 reductase (43), resulting in the accumulation of 7-dehydrocholesterol, which leads to several types of developmental defects, including mental retardation, failure to thrive, and congenital heart anomalies (44). Similarly, administration of the drug triparanol, an inhibitor of sterol-Δ-24 reductase induces the accumulation of desmosterol in plasma and tissues and produces

defects in developing rats that mimic SLOS (45). Additionally, in adult humans, triparanol administration leads to liver disease, impotence, and cataract formation (7, 8).

The basis for the pathology of these sterols is unclear. Recent data suggest that the developmental defects may be due to an interference in embryogenic processes that normally involve cholesterol. Porter, Young, and Beachy (46) reported that the vertebrate morphogenic protein, sonic hedgehog (Shh), undergoes autoproteolytic cleavage to yield a 19 kDa amino terminal signaling region that is covalently linked to cholesterol. This linkage is crucial for both the localization and signalling activity of the protein, which is responsible for the formation of segmental organizing centers in very early embryogenesis. The developmental defects observed when the Shh gene is disrupted resemble those when triparanol is administered to pregnant animals (45). One possibility to explain this similarity is that the lack of cholesterol in triparanol-treated animals leads to some Shh molecules that lack a steryl modification, which would cause inappropriate localization and signaling. Another possiblitiy is that excess levels of sterol biosynthetic intermediates could lead to the modification of Shh with these sterols, which also would be expected to cause inappropriate localization and signaling. Our findings could support and help to explain this second possibility, as they show that desmosterol has a greater tendency to desorb from membranes than does cholesterol. A similar difference in membrane affinity would be expected when comparing a demosterol-modified peptide to its cholesterol-modified counterpart. This might have a substantial effect on the diffusiblity and localization of an extracellular signaling molecule, such as Shh, in a membrane-rich tissue environment.

There has been very little study of the participation of sterol intermediates in the reverse sterol transport pathway. We hypothesize that their transport from extrahepatic cells to the liver for conversion to cholesterol could be one of the ways that animals prevent the build-up of these polar molecules in tissues. Björkhem et al. (47) have shown that only small amounts of the intermediates are found in the serum of healthy humans, not approximating the levels that appear to be produced by extrahepatic cells. This suggests the existence of an efficient system of transport and conversion to cholesterol, perhaps in the liver. Another possibility is that only a limited number of tissues accumulate large amounts of the intermediates in comparison to cholesterol. This would not diminish the importance of their removal from cells, as all tissues must develop and function properly for the health of the organism. We see that liver-derived cells possess mechanisms that assure the completion of cholesterol synthesis without significant accumulation of intermediates (4). By mediating the transport of sterol intermediates to the liver, the reverse sterol transport pathway could prevent their build-up. This would introduce an additional important function for this pathway and its ability to prevent disease.

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