Movement of plasma-membrane sterols to the endoplasmic reticulum in cultured cells

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The spontaneous turnover of plasma-membrane sterols, as measured by their transfer to the endoplasmic reticulum, was measured in quiescent cultured human skin fibroblasts and monkey arterial smooth-muscle cells. The plasma-membrane sterol pool was pulse-labelled with trace amounts of either [^3H]desmosterol or [^3H]cholesterol. We then measured the enzymic conversion of [^3H]desmosterol into [^3H]cholesterol and of [^3H]cholesterol into [^3H]cholesteryl esters in intact cells. Depending on the probe used, markedly different transfer or conversion rates were found in these cells. In quiescent human skin fibroblasts, incubated in a serum-free medium, about 1.1 % of the plasma-membrane [^3H]desmosterol was converted into [^3H]cholesterol/h, whereas in monkey arterial smooth-muscle cells the corresponding rate was 0.4 %. Under similar experimental conditions, these cells esterified less than 0.02 % (fibroblasts) and 0.12 % (smooth-muscle cells) of the plasma-membrane [^3H]cholesterol/h. The movement of sterols from the plasma membrane to the endoplasmic reticulum, as measured by the conversion of [^3H]desmosterol into [^3H]cholesterol was not blocked by colchicine, but was markedly enhanced by 3 % (w/v) dimethyl sulphoxide. In all, these results indicate that plasma-membrane sterols of cultured cells are continuously transferred to the interior of the cell at a rate substantially higher than previously appreciated. This turnover of plasma-membrane sterol molecules took place even when there was no mass transfer of sterols into the cells.

INTRODUCTION

Non-esterified cholesterol is an important structural component of cellular membranes. For reasons yet unknown, the distribution of cholesterol among the various membranes in mammalian cells is very uneven. The highest concentrations of cholesterol have been found in the plasma-membrane fraction of cells, whereas the other cellular organelles seem to contain much less cholesterol (Colbeau *et al.*, 1971; Comte *et al.*, 1976; Schroeder *et al.*, 1976; Green, 1977; Lange & Ramos, 1983). Endogenously synthesized cholesterol appears to be readily transported from the site of synthesis to the plasma membrane (DeGrella & Simoni, 1982; Lange & Matthies, 1984; Kaplan & Simoni, 1985). There is, however, no general agreement as to whether or not plasma-membrane sterol molecules are spontaneously exchanged with sterol molecules of intracellular structures. We have previously shown that exchangeable [^3H]cholesterol is available for intracellular esterification in several different cell types (Slotte & Lundberg, 1983; Slotte *et al.*, 1984a,b) and that this esterification is catalysed by acyl-CoA:cholesterol acyltransferase (ACAT; Slotte, 1987). Even though these findings clearly show that exogenously derived free [^3H]cholesterol can reach the endoplasmic reticulum, the rate at which [^3H]cholesterol is esterified may not necessarily correlate with the rate of turnover of plasma-membrane cholesterol. The regulatory state of ACAT and the rate of mixing of exogenously derived [^3H]cholesterol with endogenous cholesterol also will affect the observed ACAT-catalysed-esterification rate.

To approach the question of sterol movement between plasma membranes and intracellular membrane structures, another sterol marker, [^3H]desmosterol, was used. This sterol is converted into [^3H]cholesterol exclusively by the sterol Δ^24 -reductase, an enzyme believed to be restricted to the endoplasmic reticulum (Steinberg & Avigan, 1969; Gaylor, 1981). In the present study we therefore pulse-labelled the plasma-membrane sterol pool with trace amounts of either [^3H]desmosterol or [^3H]cholesterol and monitored their enzymic conversion into [^3H]cholesterol or [^3H]cholesteryl esters respectively.

EXPERIMENTAL

Materials

[^3H]Cholesterol (55 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA, U.S.A. [^{14}C]Oleic acid (57 Ci/mol) was from Amersham, Arlington Heights, IL, U.S.A. [^3H]Desmosterol (0.24 Ci/mmol) was generously provided by Dr. Edward H. Goh of the Section of Pharmacology, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN, U.S.A. Before use, the [^3H]desmosterol was purified to more than 98 % purity on reverse-phase t.l.c. plates. Chromatographically pure cholesterol was purchased from J. T. Baker Co. Colchicine, DL-mevalonolactone, bovine fatty-acid-free albumin and desmosterol (purity over 98 % on reverse-phase t.l.c.) were obtained from Sigma Chemicals, St. Louis, MO, U.S.A. The ACAT-inhibitory compound 58-035 {3-(decyldimethylsilyl)-N-

[2-(4-methylphenyl)-1-phenylethyl]propanamide}, was generously provided by Sandoz, East Hanover, NJ, U.S.A. The sterol Δ^24 -reductase inhibitor 20,25-diazacholesterol was generously provided by Searle Chemicals, Skokie, IL, U.S.A. Dulbecco's modified Eagle medium (MEM) and growth supplements were obtained from GIBCO. All other chemicals were of reagent grade.

Cell culture

Human skin fibroblasts were derived from skin biopsies of healthy volunteers. The cells were cultivated by conventional techniques in Dulbecco's MEM with 10% (v/v) fetal-calf serum. The cells were used between their fifth and fifteenth passage. Arterial smooth-muscle cells were derived from intima-medial explants of monkey (*Macaca nemestrina*) aorta as described by Ross (1971). Monkey arterial smooth-muscle cells were cultivated in Dulbecco's MEM with 10% calf serum. For experiments, cells were seeded at 70000 cells/35 mm-diameter dish. Cells were fed every other day (1.5 ml of growth medium/dish) and were confluent after 5–7 days.

Tracer incorporation and incubation procedures

Before experiments, confluent cells were kept for 48 h in Dulbecco's MEM with 0.2% calf serum and then an additional 24 h in serum-free medium. The cells were quiescent and non-proliferating at the time of the incubations, as verified by cell counts from separate dishes with similar treatment. To label the cellular plasma-membrane sterol pool with either [3 H]cholesterol or [3 H]desmosterol, the cells were washed once with PBS. Then 1.0 ml of serum-free medium containing 10 mM-Hepes, pH 7.4, and either [3 H]cholesterol (55 Ci/mmol, final concn. 0.2 μ Ci/ml) or purified [3 H]desmosterol (0.1 Ci/mmol, 0.2 μ Ci/ml; in ethanol) was added, and the cells were incubated in this medium for 2 h at room temperature (approx. 24 °C). After this pulse, the radioactive media were removed, the cells were washed five times with 2 ml of PBS, 1.0 ml of serum-free medium was added to each dish together with any other additions (as indicated in the Figures) and the cells were incubated at 37 °C for different periods of time. After completed incubations, the media were discarded and the cells were washed twice with 2 ml of PBS and stored frozen (–20 °C) until further analysis.

Lipid analysis

Cellular neutral lipids were extracted with hexane/propan-2-ol (3:2, v/v; Brown *et al.*, 1980). Lipid carriers (50 μ g of cholesterol, cholesteryl oleate and desmosterol/tube) were added to each tube containing the lipid extracts. Non-esterified and esterified [3 H]cholesterol were isolated on silica-gel G t.l.c. plates (Analtech, Newark, DE 19711, U.S.A.) with hexane/diethyl ether/acetic acid (270:60:3, by vol.) as the solvent. [3 H]-Cholesterol and [3 H]desmosterol were separated on Anasil RP reverse-phase plates (Analabs, North Haven, CT 06473, U.S.A.) with acetonitrile/methanol (8:1, v/v) as solvent, and identified from standard lipids run in parallel. The radioactivity in the various spots was determined by scraping the gel directly into vials to which Aquasol 2 (New England Nuclear Corp.) was added.

Other procedures

Cellular proteins remaining on dishes after lipid extraction were digested into 1.0 ml of 0.1 M-NaOH (2 h at room temperature). A portion was taken for protein determination, with bovine albumin as standard (Lowry *et al.*, 1951).

RESULTS

Esterification of plasma-membrane [3 H]cholesterol

To test for the ability of cultured cells to internalize and esterify plasma-membrane free cholesterol, the plasma-membrane cholesterol pool was labelled with trace amounts of [3 H]cholesterol by using a previously described protocol (Lange & Matthies, 1984). Under the conditions of subsequent incubations, the cells were in a quiescent state with no extracellular sterol present. Hence the movement of plasma membrane [3 H]sterols to the interior of the cells was taken to represent the spontaneous turnover of membrane sterols in the quiescent state.

With monkey arterial smooth-muscle cells, a linear increase in the cellular content of [3 H]cholesteryl esters was observed during the first 10 h of incubation (Fig. 1). During this initial phase, about 0.12% of the plasma-membrane [3 H]cholesterol was esterified/h. The radioactivity of cellular [3 H]cholesteryl esters did not further increase at longer incubations (up to 20 h). To demonstrate that this esterification of plasma-membrane

Fig. 1. Esterification of plasma-membrane [3 H]cholesterol in quiescent monkey arterial smooth-muscle cells and human skin fibroblasts

The cellular plasma-membrane cholesterol pool was labelled with trace amounts of [3 H]cholesterol, as described in the Experimental section. With monkey arterial smooth-muscle cells, one set (○) of cells received 1.0 ml of serum-free medium supplemented with 0.1% ethanol, and the other set (△) medium + the ACAT inhibitor 58-035 from Sandoz (5 μ g/ml; added in ethanol; final vol. 0.1%). The conversion of [3 H]cholesterol into [3 H]cholesteryl esters was measured as a function of time. Human skin fibroblasts were incubated in serum-free medium without additives (□). Values represent label in cellular [3 H]cholesteryl esters as a percentage of the total cellular 3 H label (means $\pm$ S.D., $n = 4$).

[^3H]cholesterol was due to the activity of ACAT, the formation of [^3H]cholesteryl esters in the presence ($5\text{ }\mu\text{g/ml}$) or absence of the potent and specific ACAT inhibitor 58-035 (from Sandoz) was measured. The formation of [^3H]cholesteryl esters in monkey arterial smooth-muscle cells was markedly inhibited by compound 58-035 (Fig. 1), suggesting the involvement of ACAT in the esterification process of non-treated cells.

Human skin fibroblasts also esterified a fraction of the [^3H]cholesterol that was derived from the plasma membrane. However, the formation of [^3H]cholesteryl esters in quiescent fibroblasts was much slower than in monkey arterial smooth-muscle cells. During a 20 h incubation in serum-free medium, human skin fibroblasts esterified less than 0.3 % of their plasma-membrane free [^3H]cholesterol (Fig. 1). This esterification activity was also inhibitable by the ACAT inhibitor 58-035 (results not shown; cf. Slotte, 1987).

Conversion of [^3H]desmosterol into [^3H]cholesterol

Since the ACAT-mediated esterification process may have been a rate-limiting step in the overall process of sterol transfer to the endoplasmic reticulum and the subsequent formation of [^3H]cholesteryl esters, we used [^3H]desmosterol as a tracer for intracellular sterol movement. Monkey arterial smooth-muscle cells were labelled with trace amounts of [^3H]desmosterol using the same protocol as for [^3H]cholesterol labelling. The conversion of [^3H]desmosterol into [^3H]cholesterol was then monitored in the cells as they were incubated in a serum-free incubation medium for different periods of time. Monkey arterial smooth-muscle cells converted plasma-membrane-derived [^3H]desmosterol into [^3H]cholesterol linearly for at least 25 h (Fig. 2). About 0.4 % of the total cellular [^3H]desmosterol was converted into [^3H]cholesterol/h. The corresponding initial conversion rate in human skin fibroblasts was approx. 1.1 %/h (Fig. 3). The conversion of [^3H]desmosterol into [^3H]cholesterol was not a spontaneous chemical reaction, since only very low levels of conversion could be observed in a cell-free system with incubation medium alone (Fig. 3). On the other hand, the conversion of [^3H]desmosterol into [^3H]cholesterol was inhibited by the compound 20,25-diazacholesterol (Fig. 2), a drug known to be a specific inhibitor of the converting enzyme sterol Δ^{24} -reductase (Winer *et al.*, 1966; Steinberg & Avigan, 1969). This indicates that the cellular conversion of plasma-membrane-derived [^3H]desmosterol was an enzyme-catalysed process.

Stimulation of the cellular esterification activity

The finding that the conversion of plasma-membrane [^3H]desmosterol into [^3H]cholesterol was much faster than the conversion of plasma-membrane [^3H]cholesterol to [^3H]cholesteryl esters indicated that the esterification reaction itself was rate-limiting under our incubation conditions. To test further this possibility, it was decided to up-regulate the cellular ACAT activity without adding exogenous sterols. We then measured the effects of this up-regulation on the esterification of plasma-membrane-derived [^3H]cholesterol. Human skin fibroblasts were incubated with increasing amounts of DL-mevalonolactone to turn on the cellular biosynthesis of cholesterol, eventually leading to an increased ACAT activity. This treatment led to a dose-related increase in [^{14}C]oleic acid incorporation into cellular cholesteryl

Fig. 2. Conversion of plasma-membrane [^3H]desmosterol into [^3H]cholesterol in cultured monkey arterial smooth-muscle cells

The cellular plasma-membrane sterol pool was labelled with trace amounts of [^3H]desmosterol, as described in the Experimental section. The cells were then incubated in serum-free medium for different periods of time, and the conversion of [^3H]desmosterol into [^3H]cholesterol was measured from the lipid extract (○). In parallel, the conversion of [^3H]desmosterol into [^3H]cholesterol was measured in cells exposed to the sterol Δ^{24} -reductase inhibitor 20,25-diazacholesterol ($0.5\text{ }\mu\text{g/ml}$) (△). Values indicate the appearance of [^3H]cholesterol (i.e. conversion from [^3H]desmosterol) in the cells (as a percentage of the total cell ^3H label) as a function of time (means $\pm$ S.D. with $n = 4$).

Fig. 3. Conversion of plasma-membrane [^3H]desmosterol into [^3H]cholesterol in cultured human skin fibroblasts

The cellular plasma-membrane sterol pool was labelled with trace amounts of [^3H]desmosterol. The cells were then incubated in serum-free medium for different periods of time, and the conversion of [^3H]desmosterol into [^3H]cholesterol was measured (○). In parallel, the conversion of [^3H]desmosterol into [^3H]cholesterol was measured in cell-free dishes with 1 ml of serum-free medium (△). Values indicate the appearance of [^3H]cholesterol (as a percentage of the total cell ^3H label) as a function of time (means $\pm$ S.D., $n = 4$).

[14 C]esters (Fig. 4), indicating that the apparent activity of ACAT was indeed increased. Concurrent with the increased [14 C]oleic acid incorporation into cholesteryl[14 C]esters, an increased esterification of plasma-membrane-derived [3 H]cholesterol was also observed (Fig. 4). These results suggest that when the ACAT activity was increased, the esterification of [3 H]cholesterol already in the vicinity of the enzyme was increased.

Fig. 4. Incorporation of plasma-membrane-derived [3 H]-cholesterol or [14 C]oleic acid into cholesteryl esters in human skin fibroblasts

The plasma-membrane cholesterol pool of quiescent human skin fibroblasts was labelled with trace amounts of [3 H]cholesterol. Cells were then incubated for 24 h in a serum-free incubation medium supplemented with either 0, 0.5, 1, 2, 5 or 10 mM-DL-mevalonolactone (added in ethanol). The cellular content of [3 H]cholesteryl esters was measured, with values (O) given as percentages of the total 3 H label found as [3 H]cholesteryl esters (means $\pm$ S.D., $n = 4$). In another set of dishes, human skin fibroblast cells were incubated for 24 h in serum-free medium with indicated amounts of DL-mevalonolactone. The cells were then exposed to 0.2 μ Ci of [14 C]oleic acid [20–30 μ M; bound to albumin (0.05–0.10 %) for 2 h]. Values (Δ) are given as cholesteryl [14 C]oleate (as c.p.m.) formed per dish (means $\pm$ S.D., $n = 4$).

Effects of different compounds on the conversion of [3 H]desmosterol into [3 H]cholesterol

To gain insight into the process of intracellular conversion of [3 H]desmosterol into [3 H]cholesterol, cells were incubated with different compounds, and their effects on the rate of conversion were measured. Because intact cells were used, it was difficult to discriminate between effects of drugs on the transport and the conversion processes; nevertheless, results proved useful. Since albumin is known to enhance the exchange of cholesterol between liposomal membranes and cell plasma membranes (Slotte & Björkerud, 1984), we wanted to test the possibility that albumin also might enhance movement of plasma-membrane [3 H]-desmosterol into the cells, possibly by acting as a carrier of lipids during endo- or pino-cytosis. Conversion of [3 H]desmosterol into [3 H]cholesterol was determined in the presence of fatty-acid-free albumin (1 mg/ml). The presence of this protein in the incubation medium did not, however, result in an increased intracellular movement/conversion of [3 H]desmosterol when compared with the situation with serum-free medium alone (Table 1).

To examine whether cytoskeletal structures were involved in the movement of plasma-membrane [3 H]-desmosterol to the conversion site, cells were incubated with 20 μ M colchicine. At this concentration, colchicine affected the gross morphological appearance of the cells as determined by optical microscopy, but this treatment did not reduce the transport/conversion in human skin fibroblasts. In fact, an increased cellular conversion of [3 H]desmosterol into [3 H]cholesterol was seen (Table 1).

Finally, by increasing the critical micellar concentration of [3 H]desmosterol with 3 % dimethyl sulphoxide (Bruckdorfer & Sherry, 1984), we were able to enhance the conversion of [3 H]desmosterol into [3 H]cholesterol in monkey arterial smooth-muscle cells by more than 40 % (Table 1).

DISCUSSION

Non-esterified cholesterol is a very dynamic molecule in the sense that it is rapidly exchanged between various

Table 1. Effects of some compounds on the conversion of [3 H]desmosterol into [3 H]cholesterol by cultured fibroblasts or arterial smooth-muscle cells

Quiescent cells in 35 mm-diameter dishes were labelled with [3 H]desmosterol as described in the Experimental section. The cells were then incubated with 1.0 ml of serum-free medium containing the indicated additions. Colchicine was added from an ethanolic stock solution, with final ethanol concentration less than 0.1 % (v/v). The appropriate control contained solvent alone. After the incubation period, cellular lipids were extracted, and the amount of [3 H]desmosterol that was converted into [3 H]cholesterol was determined. Values are given as the amount of [3 H]cholesterol (as % of total cellular label) produced from [3 H]desmosterol during the incubation period. Values are means $\pm$ S.D. ($n = 4$). Abbreviations used: FAFA, fatty-acid-free albumin; Me $_2$ SO, dimethyl sulphoxide.

Cell type	Additions	Incubation time (h)	[3 H]Cholesterol produced (%)
Human skin fibroblasts	None	24	25.03 $\pm$ 3.19 (100 %)
	FAFA (1 mg/ml)	24	23.76 $\pm$ 2.10 (95 %)
	None	20	18.02 $\pm$ 1.77 (100 %)
	Colchicine (20 μ M)	20	29.24 $\pm$ 1.52 (162 %)
Monkey arterial smooth muscle	None	23	11.34 $\pm$ 1.17 (100 %)
	Me $_2$ SO (3 %, v/v)	23	16.10 $\pm$ 1.12 (142 %)

biological lipid/protein structures (Bell, 1978). The aim of the present study was to examine whether there is a spontaneous movement of sterols from the plasma membrane to the endoplasmic reticulum of cultured cells. Previous attempts to address this question have included the measurement of the esterification of plasma-membrane-derived tracer-labelled cholesterol (Lange & Matthies, 1984; Poznansky & Czekanski, 1982). The results from such studies demonstrate that hardly any of the plasma-membrane-derived [^3H]cholesterol can be found in the esterified form, even during prolonged incubations. The conclusion has been that there is no appreciable movement of plasma-membrane cholesterol to the intracellular located esterifying enzyme. A similar conclusion was drawn from results of experiments in which the movement of [^{14}C]cholesterol from plasma membranes into cells was measured by using the cholesterol oxidase probe (Lange & Matthies, 1984).

We addressed this same question using [^3H]desmosterol as a sterol marker. This molecule differs from cholesterol only in having an extra double bond at C-24 in the acyl chain. Since desmosterol and cholesterol are metabolized similarly, but still are chemically distinguishable, desmosterol has proved to be a useful tracer in cholesterol-related metabolic studies (Long *et al.*, 1978; Gibbons & Pullinger, 1978; Pullinger & Gibbons, 1981). In experiments with cultured hepatocytes, Goh (1983) demonstrated that both [^3H]cholesterol and [^3H]desmosterol were equally valid tracers for measuring the conversion of exogenous cholesterol into intracellular metabolites. Hence the movement of plasma-membrane [^3H]desmosterol to the endoplasmic reticulum and its subsequent enzymic conversion into [^3H]cholesterol should represent the turnover of plasma-membrane sterols in general. The assumptions made here is that the converting enzyme sterol Δ^{24} -reductase is located in some intracellular membranes [probably endoplasmic reticulum (Steinberg & Avigan, 1969; Gaylor, 1981)] distinct from the plasma-membrane fraction of cells. In a very recent paper, Lange & Muraski (1987) provide evidence indicating that some of the very last steps in the biosynthetic pathway of cholesterol take place in intracellular membranes with an equilibrium buoyant density different from that of membranes containing 3-hydroxy-3-methylglutaryl-CoA reductase (sterol Δ^{24} -reductase) is located in intracellular also shown, however, that the formation of labelled cholesterol from labelled lanosterol did not take place in the plasma-membrane compartment. These results further support the assumption that the desmosterol reductase (sterol Δ^{24} -reductase) is located in intracellular membranes distinct from the cellular plasma membranes.

For the [^3H]desmosterol/[^3H]cholesterol conversion to be an index of intracellular movement, the transport process, and not the conversion step, must be rate-limiting. Pentchev *et al.* (1986) showed in mouse fibroblasts that the conversion of [^3H]desmosterol into [^3H]cholesterol was linearly proportional to the amount of desmosterol present in the incubation medium (0.5 and 5 $\mu\text{g}/\text{ml}$). This, together with the fact that human fibroblasts do not contain appreciable amounts of desmosterol, indicates that the activity of the sterol Δ^{24} -reductase is high enough not to be a rate-limiting step under the conditions used in the present study. Also, since we labelled the cellular plasma-membrane sterol pool with only trace amounts of [^3H]desmosterol, it can

safely be assumed that the capacity of cells to convert [^3H]desmosterol into [^3H]cholesterol was not exhausted.

The conversion of plasma-membrane [^3H]desmosterol into [^3H]cholesterol in both human skin fibroblasts (1.1 %/h) and monkey arterial smooth-muscle cells (0.4 %/h) was considerably higher than the conversion of plasma-membrane [^3H]cholesterol into [^3H]cholesteryl esters (0.02 %/h and 0.12 %/h for human skin fibroblasts and monkey arterial smooth-muscle cells respectively). Since these sterol species were assumed to be transferred to intracellular membrane structures with a similar rate, this discrepancy indicated that the ACAT-mediated formation of [^3H]cholesteryl esters was a rate-limiting step in the overall process. Accordingly, it was assumed that in cells there must also be a considerable movement of plasma-membrane [^3H]cholesterol to the endoplasmic reticulum (and other parts of the cell), even though it might not be esterified. When the cellular activity of ACAT was increased by incubating the cells with DL-mevalonolactone, an increased esterification of plasma-membrane [^3H]cholesterol could also be observed. This suggests that the esterification reaction indeed was rate-limiting in non-treated cells. Hence it is clear that the rate of the esterification of plasma-membrane-derived [^3H]cholesterol is not a good index for intracellular cholesterol movement.

The movement of plasma-membrane [^3H]desmosterol to the endoplasmic reticulum did not appear to be retarded by colchicine, indicating that a functioning cytoskeleton was not necessary for the transport process. Instead, it appeared that the process was mediated by simple molecular diffusion, since dimethyl sulphoxide, which increases the critical micellar concentration of sterols (Bruckdorfer & Sherry, 1984), markedly increased the movement of [^3H]desmosterol to the endoplasmic reticulum. These results are in good agreement with those of previous studies, showing that the esterification of exchangeable [^3H]cholesterol in human fibroblasts was not inhibited by colchicine (Slotte *et al.*, 1984a), but was stimulated in the presence of 2–5 % (v/v) dimethyl sulphoxide (J. P. Slotte, unpublished work).

The rate of sterol movement from the plasma membranes into the endoplasmic reticulum, as measured by [^3H]desmosterol-into-[^3H]cholesterol conversion, is slower than the movement of newly synthesized cholesterol toward the plasma membrane. From the results obtained in the present study it can be calculated that, in human skin fibroblasts, it would take about 46 h for 50 % of the plasma-membrane sterols to be transported to the endoplasmic reticulum. The corresponding number for monkey arterial smooth-muscle cells would be 125 h. These values can be compared with movement in the opposite direction, with reported half-times of 10 min (chinese-hamster ovary cells; DeGrella & Simoni, 1982) or 1–2 h (human fibroblasts; Lange & Matthies, 1984). It is conceivable that the transport of newly synthesized cholesterol to the plasma membrane is a well-regulated and fairly rapid process, whereas the movement of plasma-membrane sterols to the interior of the cells is a slower process, presumably the result of a spontaneous physical (diffusion?) process. The slowness of the process notwithstanding, such a movement of plasma-membrane sterols to the interior of the cells may be of protective importance to plasma membranes under conditions where these are exposed to cholesterol surface transfer from cholesterol-saturated lipoprotein particles.

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