Movement of 25-hydroxycholesterol from the plasma membrane to the rough endoplasmic reticulum in cultured hepatoma cells

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Abstract Oxysterols serve as both substrates and signal molecules in the cholesterol-utilizing pathways of mammalian cells. Their distribution and movement within these cells, however, have not been well characterized; therefore we have undertaken such an analysis. Radiolabeled cholesterol and 25-hydroxycholesterol were pulsed into the cell surface membranes of rat hepatoma cells and their esterification was determined. The esterification of both probes was stimulated by feeding cells lipoproteins, even though lipoprotein cholesterol might be viewed as a competitor. Unlabeled 25-hydroxycholesterol, another potential competitor, also stimulated the esterification of the cell-surface probes. Esterification of both sterols was inhibited by a variety of amphiphilic agents. This inhibition was reversed by unlabeled 25-hydroxycholesterol. In cells incubated at 15°C the fractional rate of esterification of the oxysterol was more than 100 times greater than that of cholesterol. Furthermore, the time course of esterification of plasma membrane cholesterol but not that of 25-hydroxycholesterol, was lagged. In contrast, the rate of esterification of the two probes was similar in broken cells supplied with saturating cholesterol. Finally, the transfer of 25-hydroxycholesterol from red blood cells to plasma lipoproteins was approximately 2000-fold faster than that of cholesterol. We conclude that 25-hydroxycholesterol and cholesterol are moved between the plasma membrane and endoplasmic reticulum by a common transport mechanism but that the oxysterol enters this pathway much more rapidly, possibly through a passive transfer step akin to its unmediated transfer from red cells to plasma.—**Lange, Y., J. Ye, and F. Strebel.** Movement of 25-hydroxycholesterol from the plasma membrane to the rough endoplasmic reticulum in cultured hepatoma cells. *J. Lipid Res.* 1995. **36:** 1092–1097.

Supplementary key words oxysterols • plasma membrane cholesterol

Cholesterol homeostasis utilizes a complex network of reactions involving synthesis, ingestion, storage, and consumption (1). This system appears to be controlled by the abundance of cholesterol at regulatory membrane sites (2). The abundance of cholesterol at these sites may be set by its transport into and out of those membranes (2). In particular, there is evidence for regulated circulation of sterols from the plasma membrane, their major site of accumulation, to the endoplasmic reticulum where they are esterified and where homeostatic signals to the nucleus arise (2–4). Thus, the movement of sterols between these membranes could be a central feature of cholesterol homeostasis.

Exogenous oxysterols such as 25-hydroxycholesterol (25HC) mimic excess cholesterol in this pathway. That is, they stimulate the esterification of cholesterol and inhibit its biosynthesis (5, 6). Oxysterols also are esterified (7). It has been argued that oxysterols are generated within the cell as signal molecules that index excess cell cholesterol; however, despite extensive study (8), this hypothesis has not been established definitively.

In this study, we test whether cell surface 25HC uses the same intracellular pathway as cholesterol to reach ACAT in the rough ER.

EXPERIMENTAL PROCEDURES

Materials

25-[26,27-^{3}H]hydroxycholesterol (86.1 Ci/mmol) was purchased from DuPont-New England Nuclear. [1,2-^{3}H]cholesterol (40 Ci/mmol) and [4-^{14}C]cholesterol (52 mCi/mmol) were from Amersham Corp. Oleoyl coenzyme A and 25HC were from Sigma and Steraloids (Wilton, NH), respectively.

Cells

FU5AH rat hepatoma cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (3). Blood was collected from a healthy human volunteer in 10 mM EDTA and used within 24 h.

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; ER, endoplasmic reticulum; 25HC, 25-hydroxycholesterol.

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Specific labeling of cell surfaces with sterols

Cell monolayers were rinsed and covered with buffer A (0.15 M NaCl–5 mM NaPi [pH 7.5]–1 mg/ml glucose) plus 0.05% bovine serum albumin; the radiolabeled sterols were then added in ethanol (<1% final). The flasks were incubated for 30 min at 15°C, the medium was removed, and the cells were rinsed and layered with fresh buffer A or Dulbecco's modified Eagle's medium without lipoproteins for further incubation at 37°C.

In some experiments, the cells were dissociated from the flask, washed, and suspended in buffer A. Labeled sterols in ethanol (<1%) were added to the suspension, together with 0.06% bovine serum albumin. After a 30-min incubation on ice, the cells were centrifuged, washed twice, and resuspended in buffer A. An aliquot of the cells was extracted for assay and the remainder was warmed at 37°C to allow esterification of the labeled sterols. Values were corrected for the small amount of esterification that occurred during labeling.

ACAT activity in the presence of excess exogenous cholesterol

Cell homogenates were prepared as described (3) and centrifuged for 5 min at 800 *g*. These supernatants were mixed with [^{3}H]25HC and [^{14}C]cholesterol co-solubilized in Triton WR-1339 (9, 10) and incubated for 20 min at 37°C. Bovine serum albumin (1 mg/ml), dithiothreitol (1 mM), and oleoyl coenzyme A (24 μM) were added; after 20–30 min incubation at 37°C, the samples were extracted and analyzed for the incorporation of label into esters.

Sterol transfer between red cells and plasma

Plasma and buffy coat were separated from the cells by a 5-min centrifugation at 2000 *g* at 4°C. The plasma was heated for 50 min at 56°C to inactivate lecithin:cholesterol acyltransferase (11), and centrifuged for 30 min at 15,000 rpm. The pellet and topmost layer were discarded; the clear middle region was taken for use in transfer experiments. The red cells were washed in buffer A and resuspended in this buffer plus 0.06% bovine serum albumin. Labeled sterols in ethanol (<1%) were added to the suspension and the cells were incubated for 30 min on ice, centrifuged, and washed twice in buffer A. The labeled cells were resuspended in buffer A and preincubated for 1–3 min at the desired temperature. Heat-treated plasma was added, and after various intervals, the samples were chilled and then centrifuged for 2 sec in a microcentrifuge at 4°C. Aliquots of the supernatant and input mixtures were counted for the determination of radioactivity. Transfer was corrected for the small amount of radioactivity appearing in the supernatant in the absence of plasma. No lysis of the cells occurred during the experiments.

Lipid analysis

Esters of cholesterol and 25HC were isolated by thin-layer chromatography on silica gel G plates using hexane–ethyl acetate 90:10 as solvent. 25HC ester (R_f ~0.12) migrated just above unesterified cholesterol (R_f ~0.07). 25HC remained at the origin. To improve resolution, in some assays, the TLC plate was developed for 1.4 times the interval needed for the solvent to reach the top of the

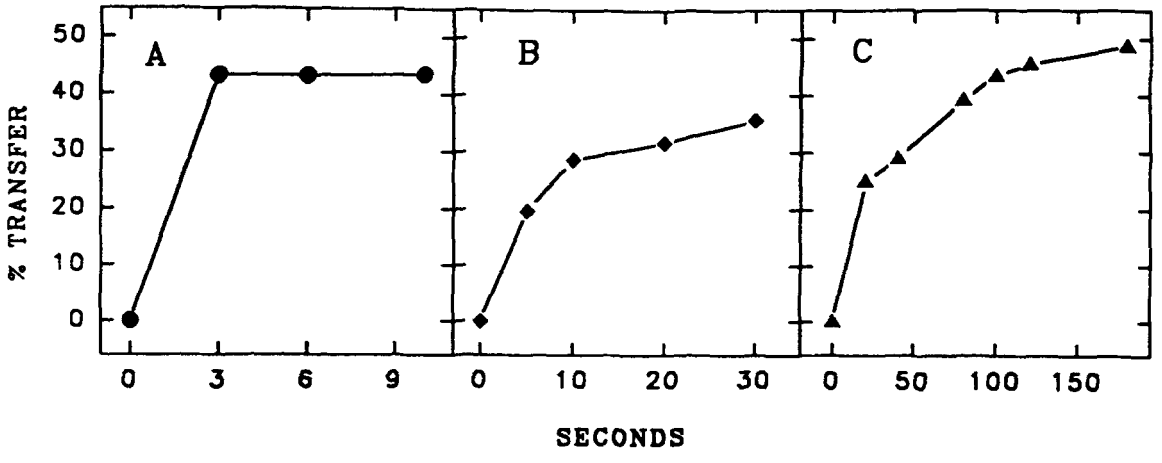

Fig. 1. The transfer of [^{3}H]25HC from red cells to plasma. Red blood cells were labeled with [^{3}H]25HC and washed as described under Experimental Procedures. Aliquots containing ~12 μg cholesterol in buffer A were incubated with heat-treated plasma containing ~10 μg cholesterol for the times and temperatures indicated. Cells were pelleted for 2 sec in a cold microcentrifuge, and the fraction of their radioactivity recovered in the resulting supernatants was determined. Transfer was corrected for release into buffer lacking plasma. Values are means of triplicates that agreed to within 10%. Panel A: 37°C; panel B, 20°C; panel C, 0°C.

plate. To confirm the identity of the 25HC ester, the gel was scraped from the plate, the lipid was extracted, saponified with ethanolic KOH, and shown by high performance liquid chromatography (12) to comigrate with authentic 25HC. Cholesterol mass was determined by high performance liquid chromatography (12).

RESULTS

Transfer of sterols from red cells to plasma

The passive transfer of cholesterol between lipid compartments proceeded with a half-time of ~2 h at 37°C (not shown but see ref. 13). In marked contrast, the movement of [^{3}H]25HC from red blood cells to plasma was too fast to be accurately determined at 37°C ($t_{1/2}$ <3 sec; **Fig. 1A**). Thus the oxysterol transferred more than 2000 times faster than cholesterol. At 20°C and 0°C, where cholesterol transfer was too slow to quantify, the half-times of transfer of 25HC were approximately 5 sec and 20 sec, respectively (Figs. 1B and 1C). In preliminary experiments, the transfer of 25HC from hepatoma cells to plasma at 0°C and 37°C proceeded with kinetics similar to those found with red cells (not shown).

Time course of esterification of plasma membrane sterols

Exogenous [^{14}C]cholesterol and [^{3}H]25HC were added to the plasma membranes of intact hepatoma cells and the time course of their esterification determined at 30°C

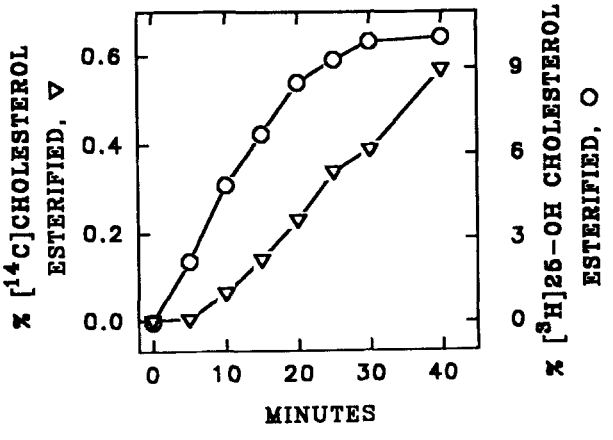

Fig. 2. Kinetics of esterification of plasma membrane 25HC compared to cholesterol at 30°C. Cells were suspended, washed, and incubated for 30 min on ice with [^{3}H]25HC (○) and [^{14}C]cholesterol (▽). The cells were washed and divided into aliquots that were either extracted immediately or incubated at 30°C for the time indicated prior to extraction and analysis for the incorporation of label into esters. Values are expressed as percent esterification of the total label (free plus esterified) corrected for the small amount of labeled esters formed during the labeling incubation.

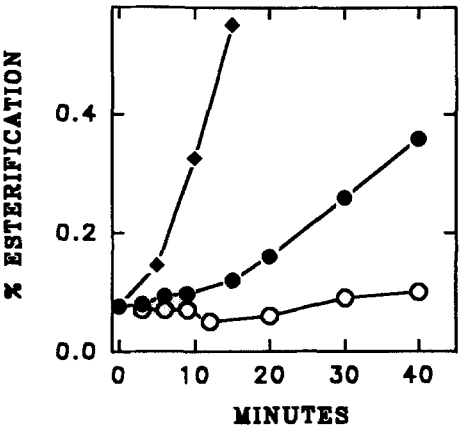

Fig. 3. Kinetics of esterification of plasma membrane cholesterol. Cells were labeled with [^{3}H]cholesterol as described in the legend to Fig. 2, and incubated at 20° (○), 30° (●), or at 37° (♦) C for the times indicated before assay of incorporation of label into cholesteryl esters. Values are expressed as percent of radioactivity esterified.

(**Fig. 2**). There were two distinctive differences between the time courses for the two probes. First, the esterification of the plasma membrane [^{14}C]cholesterol lagged while that of the [^{3}H]25HC was hyperbolic. Second, the fractional rate of esterification of the oxysterol exceeded that of the cholesterol by more than 30-fold. The plateau reached in such time courses is ascribed to the appreciable reverse reaction, cholesteryl ester hydrolysis (14). (Note that while the fractional rate of esterification was much higher for the oxysterol than for cholesterol, almost all of the sterol esterified was cholesterol.)

As incubation temperature was reduced from 37°C to 20°C, the fractional esterification of the 25HC probe remained hyperbolic in form (not shown) while that of [^{3}H]cholesterol added exogenously became increasingly lagged (**Fig. 3**).

Temperature dependence of esterification of plasma membrane sterols in vivo

The apparent rate of fractional esterification of plasma membrane [^{14}C]cholesterol and [^{3}H]25HC was determined as a function of temperature (**Fig. 4A**). For 25HC, reactions were allowed to proceed for 5 min to give initial rates and to avoid the plateau seen in Fig. 2. However, cholesterol esterification was determined after 30-min incubations to minimize the contribution of the lag. The esterification of 25HC exceeded that of cholesterol by 14-fold at 37°C. At 20°C and 15°C the esterification of the oxysterol was, respectively, 80- and 130-fold that of cholesterol (Fig. 4A, inset).

Temperature dependence of esterification of sterol probes in vitro

Homogenates of hepatoma cells were incubated with oleoyl-CoA plus the two radiolabeled sterols delivered in

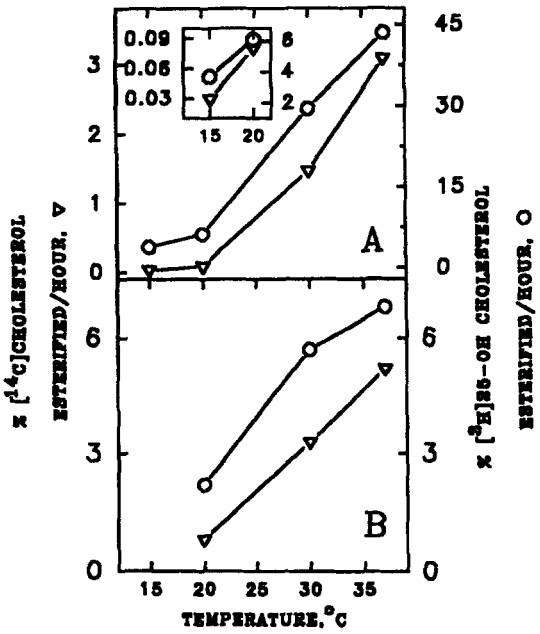

Fig. 4. Temperature dependence of esterification of cholesterol and 25HC. Panel A: Intact cells. Cells were suspended, washed, and incubated for 30 min on ice with [^{3}H]25HC (○) cosolubilized with [^{14}C]cholesterol (▽). The cells were washed and divided into aliquots that were either extracted immediately or incubated for 5 min (○) or 30 min (▽) at the indicated temperature prior to extraction and analysis for the incorporation of label into esters. Values are expressed as percent esterification of the total label (free plus esterified) per h, corrected for the small amount of labeled esters formed during the labeling preincubation. Values are means of duplicates which agreed to within 5%. The inset shows the data for 15°C and 20°C using an expanded scale. Panel B: In vitro esterification. Incubation mixtures (0.2 ml) contained cell homogenate (~14 μg cell cholesterol), 0.5 mg Triton WR-1339 bearing tracer [^{3}H]25HC, and ~28 μg [^{14}C]cholesterol, 1 mg/ml bovine serum albumin, and 1 mM dithiothreitol. The reactions, initiated with 24 μM oleoyl coenzyme A, were for 30 min at the indicated temperatures. The samples were extracted and analyzed for radiolabeled esters. The data are expressed as percent of total [^{3}H]25HC (○) and [^{14}C]cholesterol (▽) esterified per h for means of duplicates that agreed within 5%.

Triton WR-1339 (9, 10). As previously noted (9), the detergent inhibited ACAT in the absence of sterols; however, the addition of cholesterol to the Triton WR-1339 stimulated ACAT activity. The esterification of the two sterols in vitro showed a similar temperature dependence (Fig. 4B). Furthermore, the fractional velocities for the utilization of the two substrates were also similar (i.e., the ratios of esterification of 25HC to cholesterol were 1.3, 1.7, and 2.8 at 37°C, 30°C, and 20°C, respectively).

Effect of amphiphiles on the esterification of plasma membrane 25HC

Progesterone (10) and a wide range of other amphiphiles (2) appear to inhibit the movement of cholesterol from the plasma membrane to the rough ER. Progesterone and monensin (**Fig. 5**) as well as chloroquine, imipramine, nigericin, and lysophosphatidylcholine (**Fig. 6**) all inhibited the esterification of 25HC and cholesterol with

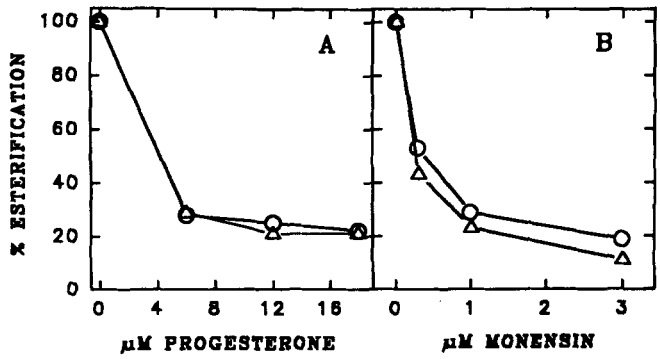

Fig. 5. The effect of progesterone and monensin on the esterification of 25HC and cholesterol. Cells were labeled with [^{3}H]25HC and [^{14}C]cholesterol as described in Fig. 2. The washed cells were preincubated for 5 min on ice with progesterone (panel A) or monensin (panel B), delivered in ethanol (<1% final), and then incubated for 40 min at 37°C prior to extraction and measurement of incorporation of label into esters; (○) 25HC; (△) cholesterol.

similar potencies. (We note that 100 μM imipramine had no effect on the sphingomyelin-induced movement of plasma membrane cholesterol to the rough ER in fibroblasts (15). This discrepancy could be due to differences in the experimental conditions or cell type.)

The addition of unlabeled 25HC partially reversed the inhibition of the esterification of both sterols by the amphiphiles (**Table 1**). The effect of the oxysterol varied between experiments, perhaps due to differences in the metabolic state of the cells; however, the reversal of the inhibition by amphiphiles was very reproducible. These data suggest that the amphiphiles do not act by inhibiting ACAT itself; 25HC is not known to stimulate the enzyme and might even be expected to exert competitive inhibition. Furthermore, the amphiphiles in Table 1 and Figs. 5 and 6 did not inhibit the esterification of either substrate in cell homogenates supplied with excess cholesterol

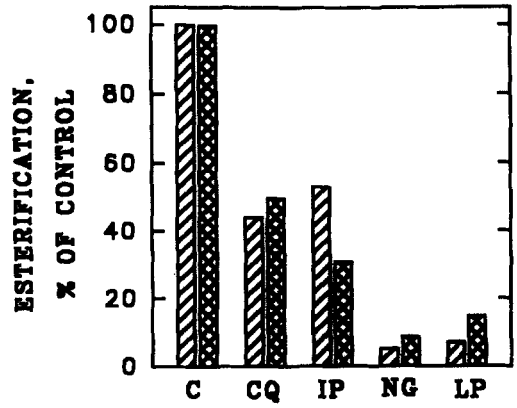

Fig. 6. The esterification of 25HC and cholesterol is inhibited in parallel by various agents. The experiment was as described in Fig. 5. C, solvent control; CQ, 70 μM chloroquine; IP, 100 μM imipramine; NG, 3 μM nigericin; LP, 30 μM lysophosphatidylcholine. Values, expressed as percent of control, are the means of duplicates which agreed to within 10%. Bar 1: 25HC; bar 2: cholesterol.

TABLE 1. 25-Hydroxycholesterol reversal of the inhibition of sterol esterification by amphiphiles

Agent (concentration)	60 μM 25HC	Esterification [³H]25HC	Esterification [¹⁴C]Cholesterol
		% of control	
None	–	100	100
None	+	149	110
Progesterone (10 μM)	–	20	19
Progesterone (10 μM)	+	39	31
Monensin (2 μM)	–	25	11
Monensin (2 μM)	+	84	51
Imipramine (70 μM)	–	42	25
Imipramine (70 μM)	+	80	42

Hepatoma cells were suspended, washed, and incubated for 30 min on ice with [³H]25HC and [¹⁴C]cholesterol co-solubilized in ethanol (<1%). The labeled cells were washed; aliquots were either extracted immediately or incubated at 37°C for 40 min with the listed agents or solvent alone. The cells then were extracted and analyzed for the incorporation of label into esters. Values were corrected for the small amount of label esterified during the preincubation and expressed as a percentage of the values for untreated cells. Values are means of duplicates that agreed to within 5%.

(**Table 2**). In contrast, Sandoz compound 58035, a well-established inhibitor of ACAT (16), effectively abolished esterification of both sterols in this system (Table 2).

Effect of cell cholesterol content on the esterification of plasma membrane 25HC

Feeding cells plasma lipoproteins stimulates their esterification of plasma membrane cholesterol (3, 17). **Table 3** shows that 25HC is under similar control: the esterification of cell surface [³H]25HC, like that of [¹⁴C]cholesterol, was twice as great in fed as in starved cells.

TABLE 2. Effect of amphiphiles on the esterification of 25-hydroxycholesterol and cholesterol in vitro

Agent (concentration)	Incorporation into Esters [³H]25HC	Incorporation into Esters [¹⁴C]Cholesterol
	% of control	
None	100	100
Progesterone (6 μM)	76	91
Monensin (1 μM)	100	94
Nigericin (3 μM)	101	97
Chloroquine (80 μM)	98	100
Lysophosphatidylcholine (15 μM)	83	89
Sandoz 58035 (1.5 μg/ml)	3	5

Aliquots of cell homogenates (containing 5–12 μg cholesterol) were incubated for 30 min at 37°C in 0.3 ml of 0.1 M Tris-HCl (pH 7.5)–0.25 M sucrose buffer containing mixtures of tracer [³H]25HC and ~25 μg [¹⁴C]cholesterol carried in 0.5 mg Triton WR-1339. The agents designated were added together with 1 mg/ml bovine serum albumin, 1 mM dithiothreitol and 24 μM oleoyl coenzyme A. After 20 min at 37°C, the samples were extracted and the incorporation of label into esters was determined. Values are means of duplicates that agreed to within 20%.

TABLE 3. Esterification of cell surface sterols in fed and starved cells

Medium	[³H]25HC	[¹⁴C]Cholesterol
	% esterification/h	
Containing lipoproteins	26.1	1.2
Lacking lipoproteins	14.4	0.7

Replicate flasks were grown to near confluence in medium supplemented with 10% fetal bovine serum. Fresh medium of the same composition or medium containing 5% lipoprotein-deficient serum was then added. After 16 h incubation, the cells were dissociated, washed, and loaded with [³H]25HC and [¹⁴C]cholesterol co-solubilized in ethanol (<1% final) for 30 min on ice. The cells were washed and either processed immediately or incubated for 20 min at 37°C prior to assay. The incorporation of the two labels into esters was determined as described in Experimental Procedures. Esterification was corrected for the small amount of esters formed during the loading step and expressed as a percent of each probe incorporated/h. Values are means of duplicate determinations that agreed within 10%.

DISCUSSION

We have contrasted radiolabeled exogenous 25HC with cholesterol as probes of intracellular sterol movement. In accord with its increased polarity compared to cholesterol, we found that the passive transfer of 25HC from red cells and hepatoma plasma membranes to plasma lipoproteins was orders of magnitude more rapid than that of cholesterol (Fig. 1). It is this higher escape potential that is presumed to account for the enhanced fractional esterification of 25HC in hepatoma cells (Figs. 2 and 4). The argument is as follows. *a)* The esterification of oxysterol is only moderately greater than that of cholesterol in cell homogenates; presumably, 25HC and cholesterol are comparable substrates for ACAT and both are delivered efficiently to the endoplasmic reticulum membranes by the detergent vehicle, Triton WR-1339 (Fig. 4B). *b)* The rate of fractional esterification of plasma membrane 25HC in intact cells is much greater than that of cholesterol, especially as the temperature is lowered (Fig. 4A). We presume that the delivery of the plasma membrane probes to ACAT in the endoplasmic reticulum is rate limiting in this case. *c)* The lag in the time course of esterification of radio-cholesterol is then taken to represent the first-order time course of labeling of the substrate pool in the endoplasmic reticulum with the plasma membrane probe. This seems to require more than 5 min at 37°C for cholesterol (Fig. 3). (That the lag does not reflect ACAT activity itself has been shown by the fact that the time course of incorporation of [¹⁴C]oleic acid into sterol esters is hyperbolic.) The absence of such a lag for the oxysterol presumably signifies its rapid equilibration between the plasma membrane sink and the endoplasmic reticulum. Therefore, a mechanism underlying these data could be the rapid passive transport of 25HC from the plasma membrane to the endoplasmic reticulum. As the transport pathway presumably has several steps, the pas-

sive transfer event would be the rate-limiting event according to this formulation. These data suggest that, compared to cholesterol, 25HC very rapidly comes to a steady-state distribution between the plasma membrane and endoplasmic reticulum.

Considerable evidence now suggests that sterol esterification is substrate-limited in vivo (2, 3, 14). Under such circumstances, the rate of sterol esterification would be governed by the ambient levels of substrate in the endoplasmic reticulum. The level of sterol in the endoplasmic reticulum appears to be set by the homeostatically controlled, bidirectional flux of the substrate between the endoplasmic reticulum and the major sources of the sterol in the plasma membrane and, perhaps, lysosomes (2). We therefore take the rate of esterification of exogenous sterol probes to reflect their transport from the plasma membrane to the endoplasmic reticulum.

The putative rapid equilibration of 25HC between the plasma membrane and endoplasmic reticulum appears to be physiologically mediated and not merely passive, for the following reasons. First, the esterification of 25HC is stimulated by feeding lipoproteins (Table 3), a homeostatic response opposite to that expected from simple competition by providing cells with excess cholesterol. Second, the esterification of 25HC by ACAT is as inhibited by agents like progesterone and monensin as is that of cholesterol itself (Figs. 5 and 6). These agents do not affect the passive transfer of 25HC from red cells to plasma lipoproteins (data not shown) nor do they inhibit ACAT activity itself (Table 2 and ref. 10). Rather, they appear to reduce the transport of sterol probes to the endoplasmic reticulum (2). It seems, therefore, that oxysterol moves from the plasma membrane to the endoplasmic reticulum not by aqueous diffusion (18) but along the pathway utilized by cholesterol, albeit at a much faster rate.

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REFERENCES

1. Goldstein, J. L., and M. S. Brown. 1990. Regulation of the mevalonate pathway. *Nature.* **343:** 425–430.
2. Lange, Y., and T. L. Steck. 1994. Cholesterol homeostasis: modulation by amphiphiles. *J. Biol. Chem.* **269:** 29371–29374.
3. Lange, Y., F. Strebel, and T. L. Steck. 1993. Role of the plasma membrane in cholesterol esterification in rat hepatoma cells. *J. Biol. Chem.* **268:** 13838–13843.
4. Wang, X., R. Sato, M. S. Brown, X. Hua, and J. L. Goldstein. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell.* **77:** 53–62.
5. Miller, S. C., and G. Melnykovych. 1984. Regulation of cholesterol biosynthesis and esterification by 25HC in a macrophage-like cell line: uncoupling by progesterone. *J. Lipid Res.* **25:** 991–999.
6. Bell, J. J., T. E. Sargeant, and J. A. Watson. 1976. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in hepatoma tissue culture cells by pure cholesterol and several of its derivatives. *J. Biol. Chem.* **251:** 1745–1758.
7. Drevon, C. A., D. B. Weinstein, and D. Steinberg. 1980. Regulation of cholesterol esterification and biosynthesis in monolayer cultures of normal adult rat hepatocytes. *J. Biol. Chem.* **255:** 9128–9137.
8. Kandutsch, A. A. 1986. Apo-B-dependent and -independent cellular cholesterol homeostasis. *In* Biochemistry and Biology of Plasma Lipoproteins. A. M. Scanu and A. A. Spector, editors. Marcel Dekker, New York. 281–300.
9. Billheimer, J. T., D. Tavani, and W. R. Nes. 1981. Effect of a dispersion of cholesterol in Triton WR-1339 on acyl-coA:cholesterol acyltransferase in rat liver microsomes. *Anal. Biochem.* **111:** 331–335.
10. Lange, Y. 1994. Cholesterol movement from plasma membrane to rough endoplasmic reticulum; inhibition by progesterone. *J. Biol. Chem.* **269:** 3411–3414.
11. Lichtman, M. A., G. V. Marinetti, and S. E. Gordesky. 1974. The effect of incubated plasma and lysolecithin on the shape and membrane lipid composition of red cells studied "in vitro". *Nouv. Rev. Fr. Hematol.* **14:** 5–24.
12. Lange, Y. 1991. Disposition of intracellular cholesterol in human fibroblasts. *J. Lipid Res.* **32:** 329–340.
13. Lange, Y., A. L. Molinaro, T. R. Chauncey, and T. L. Steck. 1983. On the mechanism of transfer of cholesterol between human erythrocytes and plasma. *J. Biol. Chem.* **258:** 6920–6926.
14. Suckling, K. E., and E. F. Stange. 1985. Role of acyl-CoA:cholesterol acyltransferase in cellular cholesterol metabolism. *J. Lipid Res.* **26:** 647–671.
15. Harmala, A., I. M. Porn, P. Mattjus, and J. P. Slotte. 1994. Cholesterol transport from the plasma membranes to intracellular membranes is inhibited by 3β-[2-(diethylamino)ethoxy]androst-5-en-17-one. *Biochim. Biophys. Acta.* **1211:** 317–325.
16. Ross, A. C., K. J. Go, J. G. Heider, and G. H. Rothblat. 1984. Selective inhibition of acyl coenzyme A:cholesterol acyltransferase by compound 58-035. *J. Biol. Chem.* **259:** 815–819.
17. Xu, X. X., and I. Tabas. 1991. Lipoproteins activate acyl-coenzyme A:cholesterol acyltransferase in macrophages only after cellular cholesterol pools are expanded to a critical threshold level. *J. Biol. Chem.* **266:** 17040–17048.
18. Johnson, W. J., F. H. Mahlberg, G. H. Rothblat, and M. C. Phillips. 1991. Cholesterol transport between cells and high-density lipoproteins. *Biochim. Biophys. Acta.* **1085:** 273–298.