Quantitation of the Pool of Cholesterol Associated with Acyl-CoA: Cholesterol Acyltransferase in Human Fibroblasts*

Yvonne Lange§ and Theodore L. Steck

From the §Department of Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612 and the ¶Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois 60637

The esterification of cholesterol in homogenates of human fibroblasts was explored as a means of estimating the size of the pool of cholesterol associated with the endoplasmic reticulum (ER) in vivo. The rationale was that the acyl-coenzyme A:cholesterol acyltransferase (ACAT) in homogenates should have access only to cholesterol associated with the (rough) ER membrane fragments in which it resides. Reacting whole homogenates to completion with an excess of [1-14C]oleoyl-CoA converted 0.1–2% of total cell-free cholesterol to [1-14C]cholesterol esters. Control studies indicated that membranes not associated with ACAT did not contribute cholesterol to this reaction.

The extent of in vitro cholesterol esterification varied with pretreatment of the cells. Exposing intact cells to serum lipoproteins, oxysterols, or sphingomyelinase increased cholesterol esterification in homogenates severalfold; exposing the cells to mevinolin or cholesterol oxidase had the opposite effect. The variation in cholesterol esterification did not correlate with either the total cellular cholesterol or the intrinsic activity of ACAT, neither of which was changed significantly by the pretreatments. Rather, the total amount of cholesterol esterified in homogenates paralleled the rate of cholesterol esterification in the corresponding intact cells. The pool of cholesterol esterified in vitro therefore appears to reflect that associated with the ER in vivo. Since several of the mechanisms keeping cell cholesterol under tight feedback control are themselves located in the ER, this pool might not only be regulated physiologically, but could, in turn, help to regulate homeostatic effector pathways.

To hold their cholesterol constant, cells regulate its biosynthesis; its uptake in LDL1; its sequestration in droplets of cholesterol esters; and, in specialized tissues, its conversion to steroid hormones, bile acids, and plasma lipoproteins (1). Cholesterol feeds back upon these processes, principally at the level of gene transcription, the synthesis and degradation of regulatory proteins, and the activities of rate-determining enzymes (acting both through their covalent modification and through the limitation of their substrate) (2, 3). How the abundance of cell cholesterol is sensed and signaled to these control elements is obscure (2).

Several of these key regulatory activities are associated with the ER and, in some cases, the associated outer nuclear membrane. These include cholesterol esterification by ACAT (4–6) and the assembly of LDL particles (7, 8), 7α-hydroxylase (9), hydroxymethylglutaryl-CoA reductase (10), the precursor forms of regulators of transcription (11, 12), and proteases that modulate some of these factors (11, 13–15). All of these proteins respond to changes in cell cholesterol. In particular, a regulated pool of cholesterol in the ER might serve as a control element in cholesterol homeostasis (16, 17).

The validation of this hypothesis calls for a demonstration that the aforementioned regulatory activities vary quantitatively with the magnitude of the ER cholesterol pool. This pool is small, however, and not easily determined (18–21). For example, a high resolution sucrose density gradient analysis indicated that the rough ER fraction contained ∼4% of cell cholesterol, but also a similar amount of plasma membrane markers (22). Correction for contamination by plasma membrane suggests that the ER cholesterol pool is negligible (5, 22, 23). This conclusion is paradoxical, given that the ER is the site of cholesterol biosynthesis, esterification, and regulation. Electron microscopic cytochemical analysis using filipin as a cholesterol stain suggested that the concentration of cholesterol in the rough ER of a mutant cell line, UT-1, was ∼3% of that in the plasma membranes (10). However, this value did not vary with the feeding of LDL to the intact cells, as expected for a regulated compartment (see below). Furthermore, quantitation of membrane cholesterol by filipin staining has limitations (18).

There is reason to consider that the small pool of cholesterol associated with the ER at the moment of homogenization might be estimated by determining its esterification in vitro with a radioactive fatty acid. Cholesterol esterification appears to be a unique attribute of the rough ER (4, 5). In addition, cholesterol esterification in vitro varies with the availability of cholesterol to the undersaturated enzyme (3, 25, 26). Furthermore, a parallel has been demonstrated between the rate of cholesterol esterification in vivo and that in the corresponding unfraccionated membranes from cells treated with LDL (6), acetylated LDL (27), progesterone (28), or oxysterols such as 25-HC (6, 29). While these results are consistent with the premise that in vitro esterification manifests the pool of cholesterol in the ER, other mechanisms could obscure this relationship. For example, there are intracellular inhibitors and activators of ACAT (30–32). Thus, the well documented activation of cholesterol esterification by exogenous 25-HC could reflect modulation of the available cholesterol pool, a direct effect on the enzyme, or both (26).

To validate the proposed assay of ER cholesterol as that...
esterified in homogenates, the following should be established. (a) ACAT activity should be uniquely associated with ER fragments. (b) Cholesterol not associated with ACAT-bearing membranes should not be esterified in the in vitro reaction. (c) All the cholesterol in the ACAT-bearing membranes should be esterified in the in vitro reaction. The assay should therefore be allowed to run to completion so that the amount of cholesterol in the pool rather than the initial rate of its esterification is determined. (d) The amount of cholesterol esterified in vitro should be discrete and vary with the rate of esterification in the parent cells under different conditions. Testing this approach was the goal this study.

EXPERIMENTAL PROCEDURES

Materials—[1,2-3H]Cholesterol (46 Ci/mmol) was from Amersham Life Science, Inc. [oleoyl-1-14C]Coenzyme A (53.3 mCi/mmol) was from DuPont NEN. LPDS was prepared from fetal bovine serum (33).

Cells—Human foreskin fibroblasts were derived from primary explants and used between passages 4 and 11 (33). The Chinese hamster ovary cell lines 25-RA and AC-29 (34) were obtained from Dr. T. Y. Chang (Dartmouth Medical School). Fibroblasts were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Chinese hamster ovary cells were grown in Ham’s F-12 medium (Sigma) containing 10% fetal bovine serum and 40 µg/ml gentamycin.

Esterification Assay—Two approaches were employed to assay cholesterol esterification in vitro. In the first, fibroblasts were washed, allowed to swell, and homogenized (5). The homogenates were cleared of large particles by low speed centrifugation, and the supernatants made 1 mM in dithiothreitol and 1 mg/ml in bovine serum albumin. The esterification reaction was started by the addition of 25 µl [14C]oleoyl-CoA. After incubation at 37 °C for the times indicated in the legends, cholesteryl esters were isolated, and their radioactivity was determined as described (5). The fraction of total cell cholesterol esterified was calculated from the radioactivity incorporated in vitro using the specific activity of the added [14C]oleoyl-CoA and the measured mass of cholesterol. In the second assay, [3H]cholesterol was added to intact cells that had been washed and suspended in ice-cold phosphate-buffered saline. The cells were incubated for 10 min on ice to allow the probe to circulate to the ER and then homogenized and assayed with unlabeled oleoyl-CoA as described above. (The time that elapsed between the addition of label and homogenization was ~30 min.) Other methods were as described in the legends and in Ref. 5.

RESULTS

The Assay—Normal human fibroblasts were prepared under different conditions known to alter their cholesterol metabolism. Their homogenates were reacted with [14C]oleoyl-CoA under optimized conditions; then, the cholesterol ester product was isolated and analyzed. Fig. 1A shows a prototypic experiment. It makes these points. (a) The esterification reaction is well described as a first-order exponential process. These are the kinetics expected for the reaction to completion of a single pool of cholesterol. This finding also supports the supposition that ACAT is far from saturated with cholesterol pool of cholesterol. This finding also supports the supposition that ACAT is far from saturated with cholesterol.

FIG. 1. Time course of cholesterol esterification in homogenates. A, esterification of endogenous cholesterol. Matched flasks of fibroblasts were incubated for 23 h with medium containing 10% serum (●) or 5% lipoprotein-deficient serum (○). The cells were dissociated, washed, and homogenized, and the time course of incorporation of [14C]oleoyl-CoA into cholesteryl esters as well as total cell cholesterol were determined as described under "Experimental Procedures." The data in both experiments were fit to first-order exponential curves with a half-time of 11.4 min. B, test for loss of ACAT activity. Homogenates were prepared, and the esterification assay was performed as described for A, except that the mixtures contained tracer [3H]cholesterol (added in 0.3% ethanol, final concentration) and 25 µl unlabeled oleoyl-CoA. For the curve on the left, [3H]cholesterol was added at time 0. For the curve on the right, aliquots of the reaction mixture were incubated for 1 h at 37 °C before [3H]cholesterol and oleoyl-CoA were added. Assays were done in duplicate and differed by 2% or less.

The results shown in Table I make these points. First, the amount of cholesterol esterified was similar to the plateau values in Fig. 1A. Second, although there was a wide variation among the results obtained in individual experiments, close agreement was observed in each experiment between the values obtained using the two labels. This finding helps to validate the method. Third, the ratio of the pairs of estimates obtained of the esterification reaction in vitro may signify the exhaustion of a small discrete pool of substrate cholesterol, presumably that present in the ACAT-bearing membrane fragments in the homogenate. (Control experiments showed that the hydrolysis of cellular cholesteryl esters was negligible during the 1-h reaction and therefore could not have contributed significantly to the pool esterified in vitro.)

We checked the method of analysis using a second approach (Table 1). Cells were prelabeled with exogenous [3H]cholesterol and then incubated to allow the surface label to circulate through the cells prior to homogenization and initiation of the in vitro cholesterol esterification described above. The same hyperbolic time course was observed for esterification of the exogenous [3H]cholesterol as obtained with [14C]oleoyl-CoA added in vitro (data not shown). We compared the extent of esterification in the two assays. In the former calculation, we used the specific activity of whole cell [3H]cholesterol since the ACAT-associated pool appears to equilibrate rapidly with that in the plasma membrane (see below). In the latter calculation, we used the specific activity of the added [14C]oleoyl-CoA, the concentration of which vastly exceeds the endogenous pool; this is the usual practice (e.g. Ref. 28).

The results shown in Table I make these points. First, the amount of cholesterol esterified was similar to the plateau values in Fig. 1A. Second, although there was a wide variation among the results obtained in individual experiments, close agreement was observed in each experiment between the values obtained using the two labels. This finding helps to validate the method. Third, the ratio of the pairs of estimates obtained
with \[^{3}H\]cholesterol and \[^{14}C\]oleoyl-CoA should, in principle, equal the degree to which the cholesterol available to ACAT had equilibrated with the plasma membrane prior to homogenization. Since this ratio was virtually unity in each experiment, the data in Table I support the premise that the pool associated with ACAT is rapidly replaced by plasma membrane cholesterol in vitro such that most of the cholesterol in the former is derived from the latter (17).

**Is Exogenous Cholesterol a Substrate for ACAT in Vitro?**—Plasma membranes bear most of the cholesterol in human fibroblasts and other mammalian cells, whereas the ER contains only a trace (5, 22, 23). If the in vitro esterification reactions shown in Fig. 1 utilized bulk cellular cholesterol, then separating ACAT from the plasma membranes should greatly reduce the plateau value for the in vitro esterification reaction. We therefore employed sucrose density gradient centrifugation to resolve ACAT from most of the more buoyant plasma membranes, as described previously (5). We then determined the pool of esterifiable cholesterol in each gradient fraction by reaction to completion with \[^{14}C\]oleoyl-CoA.

As shown in Fig. 2, the gradient profile of the cholesterol esters produced in vitro closely paralleled that of RNA, taken as a marker of the rough ER (4, 5). If ACAT had access to cholesterol in other membranes, the level of run-off cholesterol esterification would have been very high in the buoyant fractions bearing the plasma membranes. In fact, the fractional esterification of the cholesterol in the most buoyant fraction of the gradient was 12 times lower than that in the most dense fraction. Finally, the sum total of the cholesterol esterified in the gradient fractions (namely, 240 pmol) matched that in the corresponding unfractionated input (250 pmol). Since separating the bulk of the cholesterol from ACAT did not reduce the total cholesterol esterified in vitro, but rather enriched the degree of esterification in the ER fractions, the data support the premise that the assay reaction in whole homogenates utilized the small amount of cholesterol physically associated with the membranes bearing ACAT and not cholesterol in other membranes.

We also showed that washing the membranes of homogenates did not significantly reduce in vitro esterification. Thus, no soluble (e.g. transfer) factors contributed to the observed results.

Another test of whether exogenous membrane cholesterol was a substrate for ACAT was performed with mixtures of homogenates from a Chinese hamster ovary cell line, 25-RA, and a mutant line derived therefrom, AC-29, which lacked ACAT (34). We found that in mixtures of their homogenates, ACAT from the 25-RA cells esterified the \[^{3}H\]cholesterol in its own membranes, but did not esterify appreciably the radiolabel introduced into the plasma membranes of the mutant AC-29 cells (Table II). Hence, ACAT did not appear to have access to cholesterol in the other membranes in the homogenate (see also Ref. 5).

**Response of in Vitro Esterification to Pretreatments of Intact Cells**—If the plateau values for the cholesterol esterified in homogenates reflect a local physiological pool rather than a nonspecific artifact of analysis, then their magnitude should vary with appropriate pretreatments of the intact cells. To test this hypothesis, we examined the response of LDL-starved cells to the feeding of serum over prolonged time periods. As previously established, feeding cells LDL gradually increased the rate of cholesterol esterification in vivo (Fig. 3). Even though the time course of this stimulation was variable among experiments (e.g. compare A and B), the rate of esterification in vivo was closely paralleled by the total amount of cholesterol esterified in the corresponding homogenate. Typically, overnight incubation of cells with serum increased the esterification of cholesterol in vitro ~4-fold (Table III), and purified human LDL elicited a similar response (data not shown).

The amount of cholesterol esterified in the in vitro run-off assay also varied characteristically with other pretreatments of intact cells. Two treatments known to stimulate esterification of cholesterol in intact cells had the corresponding effect on run-off esterification in vitro (Table III); these were 25-HC (Ref. 29) and sphingomyelinase (see Refs. 35 and 36). Preincubation of cells with mevinolin, an inhibitor of cholesterol biosynthesis (37), or the oxidation of a small fraction of the plasma membrane cholesterol of intact cells with cholesterol oxidase, decreased the amount of cholesterol esterified in vitro (Table III).

To verify that the variation in cholesterol esterification in Table III was not a reflection of enzyme activity, we determined ACAT levels in the presence of excess cholesterol added in vitro as a Triton WR-1339 solution (38). The exogenous cholesterol stimulated esterification as much as 4-fold. Furthermore, it abolished the differences in ACAT activity among the homogenates of cells that had been fed LDL or starved or incubated with 25-HC as in Table III (data not shown). These data support the premise that the wide variation in the run-off values for cholesterol esterification in vitro reflects substantial differences in the availability of cholesterol to a fairly constant level of undersaturated cellular ACAT (3).
Endoplasmic Reticulum Cholesterol

Cells of a Chinese hamster ovary cell line (25-RA) and a derived mutant cell line lacking ACAT activity (AC-29) were suspended and washed; a portion of each was labeled with \(^{3}H\)cholesterol, and homogenates were prepared as described in the legend to Table I. Roughly equivalent aliquots of labeled and unlabeled homogenates (measured as cholesterol mass) were mixed. Esterification was measured as described under “Experimental Procedures”; an incubation time of 10 min was used to obtain initial rates. Total cell cholesterol mass was determined under “Experimental Procedures”; an incubation time of 10 min was used to obtain initial rates. Total cell cholesterol mass was determined

**TABLE II**

| Labeled homogenate | Unlabeled homogenate | \(^{3}H\)Cholesterol esterified |
|--------------------|----------------------|-------------------------------|
| Wild type          | Wild type            | 0.93                          |
| Wild type          | Mutant               | 0.32                          |
| Mutant             | Mutant               | 0.01                          |
| Mutant             | Wild type            | 0.02                          |

We also considered whether the esterification reaction in homogenates varied with the total cell cholesterol level as opposed to a special (ER) pool. We found that eliminating 5–7% of cell cholesterol (Table III). Feeding serum overnight greatly increased total cholesterol esterification in homogenates (Table III), although it decreased total cell cholesterol slightly (Table IV). (Total cell cholesterol was apparently held constant by homeostatic responses such as esterification.) Even more dramatically, 25-HC causes a significant decrease in cell free cholesterol (29) under conditions where it increases the run-off pool in vitro (Table III). Presumably, this is because 25-HC stimulates the transfer of cholesterol from plasma membranes to the ER, thereby promoting its esterification (16).

**DISCUSSION**

These results support the premise that the plateau values in our in vitro run-off esterification assay provide a quantitative measure of a discrete compartment of cholesterol associated with ACAT in vivo. It is reasonable to consider that the pool in question is associated with the ER in the intact cell. For one thing, ACAT appears to be confined to the (rough) ER (4–6). Furthermore, the run-off pool does not seem to draw upon the much larger store of cholesterol in the plasma membrane. This conclusion is based upon several lines of evidence. (a) The cholesterol pool that was esterified in vitro was small and finite (Fig. 1). (b) It was not diminished, but rather enriched by the separation of the ER from plasma membrane fragments (Fig. 2). (c) There is no evidence in the literature that cell homogenization brings about the transfer of plasma membrane cholesterol to ER fragments. Indeed, ER cholesterol is undetectable in homogenates analyzed by subcellular fractionation (see the Introduction). (d) Because the passive transfer of cholesterol between membranes is very limited in vitro (40), microsomal redistribution should not be appreciable during our assays. Evidence against such passive transfer is provided in Fig. 2 and Table II (a). It would be difficult to explain why the plateau values vary dramatically and predictably with manipulations of the cell prior to homogenization (Table III) if the esterified pool were an in vitro artifact. Indeed, we can take as an upper bound on the amount of extraneous cholesterol esterified in
Cells were incubated for 18 h in medium supplemented with 5% LPDS or 10% serum before being suspended and washed. Their lipids were extracted, and free cholesterol, cholesteryl esters, and cell protein were determined (5). Values are averages of duplicate or triplicate determinations that agreed to within 3%.

| Addition | Cholesterol | Cholesteryl esters |
|----------|-------------|--------------------|
|          | \( \mu g / m g \) protein | \( \mu g / m g \) protein |
| Exp. 1   |             |                    |
| LPDS     | 43.9        | 1.6                |
| 10% serum| 40.3        | 6.9                |
| Exp. 2   |             |                    |
| LPDS     | 61.3        | 2.8                |
| 10% serum| 59.7        | 10.3               |

The lowest values obtained in Table III. This is \( \approx 0.1\% \) of total cell cholesterol; however, the actual value for the esterification of contaminating cholesterol may have been much lower than this.

On the other hand, we cannot specify the precise location of the pool of cholesterol esterified in the run-off assay. If ACAT is confined to the rough ER (4–6), the smooth ER and nuclear envelope might not be sampled by the assay. Moreover, the rough ER might itself be compartmentalized (41). Furthermore, the cholesterol pool in question might not actually reside in the ER proper, but rather in (shuttle) membranes that remain in a stable association with the ER following homogenization. In addition, when cytochemical staining with filipin was used to analyze intracellular cholesterol distribution in the mutant cell line UT-1, it was neither the smooth nor the rough ER, but the novel crystallloid ER and its associated cisternal membranes (not observed in the wild type) whose cholesterol levels responded to the feeding of LDL to cells (10). Thus, at this point, we interpret the run-off pool as a functional rather than an anatomical compartment.

We found that 0.1–2% of cellular cholesterol was available to ACAT in vitro. The value varied with physiological and experimental manipulations (Fig. 3 and Table III). A recalibration of earlier data (e.g. Ref. 29) gives similar values, even though those studies sought rather different information and inferred initial rates of esterification rather than compartment size. Furthermore, the early reports did not examine whether all of (or only) the ER cholesterol pool was esterified. Such uncertainty may explain why the early data have not been employed as a quantitative measure of ER pool size heretofore.

A recent study reported “a significant positive correlation between ACAT activity and free cholesterol in mouse liver microsomes” (24). However, the level of cholesterol in those microsomal preparations was high and could well have resided in plasma membrane fragments, which are typically abundant in microsomes (22). Our data (Fig. 2 and Table II) suggest that the plasma membrane cholesterol in those microsomes should not have served as a substrate for ACAT. These considerations may explain why the correlation observed in that study (24) was weak as compared, for example, with the close parallel between the in vivo and in vitro assays seen in Fig. 3. We suggest that run-off esterification is a better indicator of ER cholesterol than is the microsomal quotient.

It could be argued that the direct activation of ACAT by 25-HC (28) explains its ability to stimulate cholesterol esterification in vitro (Table III) (29). Several lines of evidence, however, argue against direct enzyme activity in our system. (a) Such activation of ACAT by 25-HC has been seen only when cell cholesterol levels are unusually low (26). (b) The addition of 25-HC to control homogenates had a minimal effect on cholesterol esterification, even when the cells had been starved of cholesterol or when excess cholesterol was added to the homogenate (data not shown). (c) Just as seen for cholesterol feeding in vivo, pretreatment with 25-HC in vitro increased the extent of esterification in vitro (i.e. the size of the available cholesterol pool) rather than the fractional rate of esterification, as would be the case for enzyme activation. (d) 25-HC was removed from our cells prior to homogenization; in some experiments, we also washed the membranes before the assay. (e) There is independent evidence that 25-HC acts to increase ER cholesterol in intact cells (16).

Our data therefore support strongly the working hypothesis that in vitro run-off cholesterol esterification makes manifest a regulated ER pool. Clearly, the cholesterol in this compartment does not vary with total cell cholesterol; it could instead be a measure of the ambient excess of cellular cholesterol over a physiological threshold value (42). The large changes we observed in the run-off pool with minimal changes in total cell cholesterol support such a threshold mechanism. The presumed function of physiological modulation of ER cholesterol would be to control the activity of the various regulatory elements located in the ER so as to maintain cellular cholesterol homeostasis (17). While we cannot rule out the participation of other cellular pools or mechanisms (30, 31), our hypothesis is, at least, parsimonious.

Since almost all of the cholesterol in animal cells is located in the plasma membrane and associated membranes (43), the size of the ER pool might be particularly or uniquely responsive to the abundance of cholesterol in those membranes. The mechanism defining the magnitude of the ER cholesterol pool could involve the regulated circulation of cholesterol between the plasma membrane and the ER (17). The cellular responses to LDL, 25-HC, sphingomyelinase, cholesterol oxidase, and other effectors (16) might then be mediated through the dynamic representation of plasma membrane cholesterol in the ER. For example, it has been shown that the threshold for homeostatic responses of cells to their cholesterol load is influenced by the level of sphingomyelin and other phospholipids in their plasma membranes (44). A threshold effect would then explain why the hydrolysis of a minute amount of plasma membrane sphingomyelin (35) greatly increases the apparent level of ER cholesterol (Table III).

If the entire cell cholesterol pool circulates through the ER approximately once per h (5) when the ER pool is \( \approx 1\% \) of the total, the ER compartment would be flushed out and thus reset by plasma membrane cholesterol in \( \approx 1\) min. Such rapid turnover is also an implication of the results in Table I. Those data suggest that complete equilibration of labeled cholesterol between the plasma membrane and the ER of intact cells could have occurred on ice during the \( \approx 30\) min that elapsed between the addition of the label and the homogenization of the cells. Rapid equilibration with the ER could serve to fine-tune plasma membrane cholesterol homeostasis.

Finally, the assay described above could contribute to the analysis of the regulation and integration of the various cholesterol control elements in the ER of intact cells. For example, whether the action of plasma membrane phospholipids in regulating cholesterol homeostasis (35, 42, 44) is through the adjustment of ER cholesterol might now be explored systematically. In addition, cysteine proteases that regulate ER proteins controlling cholesterol homeostasis are thought to be themselves regulated by cholesterol; among the regulated ER proteins are sterol regulatory element-binding proteins (14, 15), hydroxymethylglutaryl-CoA reductase (13), and a putative labile inhibitor of ACAT activity (30, 31). Our assay can be used to determine whether or not the action of these proteases varies with the cholesterol in the ER compartment. Similarly, it is presently uncertain whether oxysterols act directly on regula-
tory ER proteins such as ACAT (26) and the cysteine proteases (11) or, as suggested by the results in Table III, modulate their activity through the level of ER cholesterol (see Ref. 16). Finally, Skiba et al. (45) have identified a set of treatments of cells that affect their esterification of cholesterol, but not ACAT itself; it can now be established whether any of these treatments acts through the alteration of the ER-associated cholesterol pool. These applications will serve to put our assay to the test.

Acknowledgments—The Chinese hamster ovary cell lines 25-RA and AC-29 were the generous gift of Dr. T.-Y. Chang. We are grateful to Jin Ye for expert assistance.

REFERENCES

1. Fielding, C. J., and Fielding, P. E. (1985) in Biochemistry of Lipids and Membranes (Vance, D. E., and Vance, J. E., eds) pp. 404–474, Benjamin/Cummings, Menlo Park, CA.
2. Goldstein, J. L., and Brown, M. S. (1990) Nature 342, 425–430.
3. Suckling, K. E., and Stange, E. F. (1985) J. Biol. Chem. 260, 10463–10473.
4. Reinhart, M. P., Billheimer, J. T., Faust, J. R., and Gaylor, J. L. (1987) J. Biol. Chem. 262, 9649–9655.
5. Lange, Y., Strebel, F., and Steck, T. L. (1993) J. Biol. Chem. 268, 13838–13843.
6. Chang, C. C. Y., Chen, J., Thomas, M. A., Cheng, D., Del Priore, V. A., Newton, R. S., Pape, M. E., and Chang, T.-Y. (1995) J. Biol. Chem. 270, 29532–29540.
7. Borchardt, R. A., and Davis, R. A. (1987) J. Biol. Chem. 262, 16394–16402.
8. Boren, J., Graham, L., Wietesten, M., Scott, J., White, A., and Olofsson, S.-O. (1992) J. Biol. Chem. 267, 9858–9863.
9. Russell, D. W., and Setchell, K. D. R. (1992) Biochemistry 31, 4737–4749.
10. Orci, L., Brown, M. S., Goldstein, J. L., Garcia-Segura, L. M., and Anderson, R. G. W. (1984) Cell 36, 835–845.
11. Wang, X., Sato, R., Brown, M. S., Hua, X., and Goldstein, J. L. (1994) Cell 77, 53–62.
12. Hua, X., Sakai, J., Ho, Y. K., Goldstein, J. L., and Brown, M. S. (1995) J. Biol. Chem. 270, 29242–29247.
13. McGee, T. P., Cheng, H. H., Kumagai, H., Omura, S., and Simoni, R. D. (1996) J. Biol. Chem. 271, 25630–25638.
14. Hua, X., Sakai, J., Brown, M. S., and Goldstein, J. L. (1996) J. Biol. Chem. 271, 10379–10384.
15. Hua, X., Nohrufftii, A., Goldstein, J. L., and Brown, M. S. (1996) Cell 87, 415–426.
16. Lange, Y., and Steck, T. L. (1994) J. Biol. Chem. 269, 29371–29374.
17. Lange, Y., and Steck, T. L. (1996) Trends Cell Biol. 6, 205–208.
18. Reinhart, M. P. (1990) Experientia (Basel) 46, 599–611.
19. van Meer, G. (1993) Curr. Opin. Cell Biol. 5, 661–673.
20. Allan, D., and Kallen, K.-J. (1993) Prog. Lipid Res. 32, 195–219.
21. Liscum, L., and Underwood, K. W. (1995) J. Biol. Chem. 270, 15443–15446.
22. Beaufay, H., Anez-Costescu, A., Thines-Sempoux, D., Who, M., Robb, M., and Berthet, J. (1974) J. Cell Biol. 61, 213–231.
23. Lange, Y., Swaisgood, M. H., Ramos, B. V., and Steck, T. L. (1989) J. Biol. Chem. 264, 3786–3783.
24. Uchneen, P. J., Oka, R., Sullivan, M., Chang, C. C. Y., Chang, T. Y., and Chan, L. (1995) J. Biol. Chem. 270, 26192–26201.
25. Duolittle, G. M., and Chang, T.-Y. (1982) Biochim. Biophys. Acta 713, 529–537.
26. Chang, D., Chang, C. C. Y., Qu, X. M., and Chang, T.-Y. (1995) J. Biol. Chem. 270, 685–695.
27. Brown, M. S., Goldstein, J. L., Krieger, M., Ho, Y. K., and Anderson, R. G. W. (1979) J. Cell Biol. 82, 597–613.
28. Goldstein, J. L., Faust, J. R., Dugos, J. H., Corravat, R. J., and Brown, M. S. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 1877–1881.
29. Brown, M. S., Dana, S. E., and Goldstein, J. L. (1975) J. Biol. Chem. 250, 4025–4027.
30. Tabas, I. (1995) Curr. Opin. Lipidol. 6, 260–268.
31. Schissel, S. L., Beatin, N., Zha, X., Maxfield, F. R., and Tabas, I. (1995) Biochemistry 34, 10463–10473.
32. Axelsson, M., and Larsson, O. (1995) J. Biol. Chem. 270, 15102–15110.
33. Lange, Y., and Matthes, H. G. (1984) J. Biol. Chem. 259, 14624–14630.
34. Cadigan, K. M., Heider, J. G., and Chang, T.-Y. (1988) J. Biol. Chem. 263, 274–282.
35. Slote, J. P., and Bierman, E. L. (1988) Biochem. J. 260, 653–658.
36. Peri, I. M., and Slote, J. P. (1995) Biochem. J. 306, 269–274.
37. Kwanen, P. T., Bilheimer, D. W., Goldstein, J. L., Jaramillo, J. J., and Brown, M. S. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 1194–1198.
38. Lange, Y. (1994) J. Biol. Chem. 269, 3411–3414.
39. Smart, E. J., Ying, Y.-S., Conrad, P. A., and Anderson, R. G. W. (1994) J. Cell Biol. 127, 1185–1197.
40. Steck, T. L., Kezdy, F. J., and Lange, Y. (1988) J. Biol. Chem. 263, 13023–13031.
41. Vertel, B. M., Walters, L. M., and Mills, D. (1994) Curr. Cell Biol. 3, 325–341.
42. Xu, X.-X., and Tabas, I. (1991) J. Biol. Chem. 266, 17040–17048.
43. Lange, Y. (1992) J. Biol. Chem. 267, 13392–15110.
44. Okwu, A. K., Xu, X.-X., Shiratori, Y., and Tabas, I. (1994) J. Biol. Chem. 269, 15443–15446.
45. Skiba, P. J., Zha, X., Maxfield, F. R., Schissel, S. L., and Tabas, I. (1996) J. Biol. Chem. 271, 13392–13460.