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ROLE OF THE PLASMA MEMBRANE IN THE MECHANISM OF CHOLESTEROL EFFLUX FROM CELLS

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In order to investigate the role of the plasma membrane in determining the kinetics of removal of cholesterol from cells, the efflux of [^{3}H]cholesterol from intact cells and plasma membrane vesicles has been compared. The release of cholesterol from cultures of Fu_5AH rat hepatoma and WIRL-3C rat liver cells to complexes of egg phosphatidylcholine (1 mg/ml) and human high-density apolipoprotein is first order with respect to concentration of cholesterol in the cells, with half-times ($t_{1/2}$) for at least one-third of the cell cholesterol of 3.2 ± 0.6 and 14.3 ± 1.5 h, respectively. Plasma membrane vesicles (0.5–5.0 μm diameter) were produced from both cell lines by incubating the cells with 50 mM formaldehyde and 2 mM dithiothreitol for 90 min. The efflux of cholesterol from the isolated vesicles follows the same kinetics as the intact, parent cells: the $t_{1/2}$ values for plasma membrane vesicles of Fu_5AH and WIRL cells are 3.9 ± 0.5 and 11.2 ± 0.7 h, respectively. These $t_{1/2}$ values reflect the rate-limiting step in the cholesterol efflux process, which is the desorption of cholesterol molecules from the plasma membrane into the extracellular aqueous phase. The fact that intact cells and isolated plasma membranes release cholesterol at the same rate indicates that variations in the plasma membrane structure account for differences in the kinetics of cholesterol release from different cell types. In order to investigate the role of plasma membrane lipids, the kinetics of cholesterol desorption from small unilamellar vesicles prepared from the total lipid isolated from plasma membrane vesicles of Fu_5AH and WIRL cells were measured. Half-times of cholesterol release from plasma membrane lipid vesicles of Fu_5AH and WIRL cells were the same, with values of 3.1 ± 0.1 and 2.9 ± 0.2 h, respectively. Since bilayers formed from isolated plasma membrane lipids do not reproduce the kinetics of cholesterol efflux observed with the intact plasma membranes, it is likely that the local domain structure, as influenced by membrane proteins, is responsible for the differences in $t_{1/2}$ values for cholesterol efflux from these cell lines.

Introduction

Previous studies from this laboratory have established that the mechanism of cholesterol efflux from cells involves diffusion of cholesterol molecules which have desorbed from the plasma membrane through the aqueous phase until they collide with, and are absorbed by, acceptor par-

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PC, phosphatidylcholine.

ticles which transport the cholesterol away from the cells [1,2]. The rate of cholesterol removal is a function of the acceptor concentration and, in the region where cholesterol efflux is zero-order, the rate-limiting step in the transport process is desorption of cholesterol from the plasma membrane into the extracellular medium. This mechanism of cholesterol exchange or transfer via aqueous diffusion has been established for model membrane systems [3,4], serum lipoproteins [5] and various cell systems [6–9].

We have found previously that different cells, such a fibroblasts and rat hepatomas, release cholesterol to a given acceptor at different rates. This occurs under conditions where desorption from the plasma membrane is rate-limiting, so that the variations in $t_{1/2}$ should be a reflection of the properties of the plasma membrane of the donor cells [2]. We have now explored these effects in more detail, by comparing cholesterol efflux from intact cells and plasma membrane vesicles from the same cells. Plasma membrane vesicles and intact parent cells release cholesterol at the same rate, indicating that the structure of the plasma membrane can be an important determinant of the rate at which cholesterol leaves cells to extracellular acceptor particles.

Materials and Methods

Cells

The Fu_5AH rat hepatoma cell line was originally derived from a Reuber H-35 rat hepatoma [10,11]. The WIRL-3C rat liver cell line was obtained from Dr. L. Diamond, Wistar Institute, and has been maintained in culture since 1973 [12]. Stock monolayers of cells were grown in Eagle's minimal essential medium supplemented with Eagle's basal medium vitamins, 50 U/ml of penicillin, 50 μg/ml of streptomycin and 5% calf serum. Cells were grown at 37 °C in an atmosphere of 5% CO_2/95% air. All cultures were free of mycoplasma as demonstrated by routine screening. To prelabel cells with [^{3}H]cholesterol, monolayers approx. 50–75% confluent in 100 mm Petri dishes were washed three times with serum-free medium and refed with medium supplemented with 1% calf serum, solvent-extracted delipidized calf serum protein (2.5 mg/ml) [13], dipalmitoylphosphatidylcholine (8 μg/ml), unesterified cholesterol (4 μg/ml), and [7(n)-^{3}H]cholesterol (0.5 μCi/ml of medium, 21.5 Ci/mmol). The phospholipid and cholesterol were dissolved in a small volume of ethanol (0.05% of final volume) and were added to the protein-containing medium. Labeling of the cells with the [^{3}H]cholesterol occurred during a 3 day incubation period. We have previously observed that this labeling procedure does not elicit the deposition of esterified cholesterol in these cells and that less than 5% of the cell cholesterol is esterified [2].

Plasma membrane vesicles

Plasma membrane vesicles were produced using a modification of the published procedures previously used for a variety of other tissue culture cells [14,15]. Cell monolayers, prelabeled with [^{3}H]-cholesterol, were washed four times with phosphate-buffered saline, after which the monolayers were exposed for 90 min at 37 °C to 50 mM formaldehyde and 2 mM dithiothreitol in phosphate-buffered saline. Following this incubation period, the buffer containing some plasma membrane vesicles was collected and the monolayers were then washed with hypertonic phosphate-buffered saline (0.44 M NaCl, pH 7.6). This washing procedure released large numbers of vesicles, which were pooled with those previously collected. The vesicle preparations were centrifuged at 750 × *g* for 10 min to remove contaminating whole cells, after which the supernatant was centrifuged at 25 000 × *g* for 30 min at 4 °C in a Beckman 60 Ti rotor. The pelleted vesicles were resuspended in Ca^{2+}/Mg^{2+}-free minimal essential medium buffered with Hepes, supplemented with 1 mg/ml albumin.

HDL apolipoprotein / PC complexes

HDL apolipoprotein/egg phosphatidylcholine complexes were prepared using HDL apolipoprotein as previously described [2]. These complexes, which were used to promote cholesterol efflux, were added to suspensions of whole cells or plasma membrane vesicles at a final concentration of 1 mg PC/ml. This concentration has previously been shown to stimulate maximal cholesterol efflux from cells [2].

Measurement of cholesterol efflux

(a) Intact cells and plasma membrane vesicles. For efflux experiments, monolayers labeled with [^{3}H]cholesterol were washed three times with phosphate-buffered saline containing 0.75 mM $CaCl_2$ and 0.5 mM $MgCl_2$. Cells were detached by exposure to Ca^{2+}/Mg^{2+}-free phosphate-buffered saline supplemented with 1% glucose and 0.2% EDTA. Cells were washed once by centrifugation in Ca^{2+}/Mg^{2+}-free minimal essential medium, buffered with 12.5 mM Hepes. The washed cell pellets were resuspended in the above medium supplemented with albumin (1 mg/ml).

To initiate the experiment, either whole cell or plasma membrane vesicle suspensions were mixed with HDL apolipoprotein/PC complexes to yield a final concentration of 1 mg PC/ml and from 2.5–5.0 μg/ml cell or plasma membrane vesicle free cholesterol. The suspension medium consisted of Hepes-buffered Ca^{2+}/Mg^{2+}-free minimal essential medium containing 1 mg/ml albumin. 2 ml of suspension medium were incubated at 37°C on a rotary shaker at 125 rpm. At intervals, duplicate 150-μl aliquots were removed, cooled on ice to 4°C, and the whole cells or plasma membrane vesicles were pelleted by centrifugation in a 42.2 Ti Beckman rotor at 114000 × g for 15 min at 4°C. The release of [^{3}H]cholesterol to acceptors was quantitated by liquid scintillation techniques. At both the beginning and end of the incubation period, aliquots were taken to establish the total [^{3}H]cholesterol in the suspension mixture. The [^{3}H]cholesterol efflux data were analyzed as described previously [1,2].

(b) Small unilamellar vesicles. [^{14}C]Cholesterol exchange or transfer between donor and acceptor small, unilamellar vesicles prepared by sonication as described below was monitored essentially as described previously [3], using ion-exchange chromatography to separate the two vesicles populations. In all experiments, neutral acceptor vesicles were prepared from egg PC with 10 mol% cholesterol and a trace of cholesteryl[^{3}H]oleate to monitor recovery [3]. Negatively charged donor vesicles were prepared either from the total lipid extract of plasma membrane vesicles or from the phospholipid extracted from whole cells. There was sufficient acidic lipid in both of these preparations to ensure effective binding of the donor small unilamellar vesicles to the DEAE-Sepharose CL-6B column (< 2% leakage), thereby achieving a good separation from the neutral small unilamellar vesicles. [^{14}C]Cholesterol in trace amounts was added to the donor vesicles which had been prepared from plasma membrane vesicle total lipid. The cholesterol/phospholipid molar ratios of the cellular membrane lipids were 0.48 for Fu_5AH and 0.39 for WIRL membranes (Table I). Donor small unilamellar vesicles prepared from whole cell phospholipids contained 10 mol% unlabeled cholesterol in addition to the trace of [^{14}C]-cholesterol.

The small unilamellar vesicles were prepared by sonication of the lipids at 4°C using a Branson 350 sonifier with a tapered microtip at setting 3 for 20 min. The resulting dispersions were centrifuged at 4°C in a Beckman 40 rotor at 70000 g_{max} for 1 h to precipitate titanium and any poorly dispersed lipid. Donor and acceptor small unilamellar vesicles were incubated in a 1 : 10 w/w ratio (0.01–0.1 : 0.1–1.0 mg lipid/ml) at the desired temperature. Transfer of [^{14}C]cholesterol was followed by removing aliquots at different times and quickly separating donor and acceptor vesicles on DEAE-Sepharose CL-6B columns pre-equilibrated with egg PC vesicles. The kinetics of [^{14}C]cholesterol transfer were analyzed as described previously [3].

Analytical procedures

Proteins were determined by the sodium dodecyl sulfate-Lowry procedure [16]. Lipids were extracted using the methods of Bligh and Dyer [17]. Cholesterol was quantitated using gas-liquid chromatography techniques [18]. Phospholipid phosphorus was measured following the procedure of Sokoloff and Rothblat [19]. ^{3}H and ^{14}C were quantitated by liquid-scintillation techniques in a Beckman LS-7500 counter using Scintiverse [3]. Assays for 5′-nucleotidase were conducted using a modification of the procedure described by Widnell and Unkeless [20].

Materials

Cholesterol, dipalmitoylphosphatidylcholine, egg phosphatidylcholine, dithiothreitol and albumin (essentially fatty acid-free) were purchased from Sigma (St. Louis, MO). Radiolabeled sterols

were purchased from Amersham (Arlington Heights, IL) and repurified by thin-layer chromatography immediately prior to use. Tissue culture supplies were obtained from Flow Labs (McLean, VA). Solvents, scintillation fluid, thin-layer plates and formaldehyde were purchased from Fisher Scientific (King of Prussia, PA). Delipidized serum protein was prepared as previously described [13].

Cellular phospholipids were separated from the total lipid extract by thin-layer chromatography using silica gel G plates developed in petroleum ether/ethyl ether/acetic acid (75 : 25 : 1, v/v). The phospholipid band was scraped into a conical centrifuge tube to which was added chloroform/methanol/acetic acid/water (50 : 50 : 5 : 10, v/v). Following centrifugation to pellet the silica gel, the supernatant was removed and the gel washed once with the same solvent mixture. The supernatants were pooled and water (0.26 × vol.) was added to produce a phase separation. Phospholipids were quantitatively recovered in the lower chloroform phase.

Results

It has been demonstrated that exposure of cells to formaldehyde results in the formation of plasma membrane vesicles which can then be collected from the incubation medium (see Refs. 14 and 15). Preliminary studies using Fu_5AH and WIRL cells demonstrated that 50 mM formaldehyde and 2 mM dithiothreitol produced extensive vesiculation in both cell types. It was observed, however, that, following a 90 min incubation period, a large number of vesicles remained attached to the cell monolayer. Washing the monolayers with alkaline buffer (pH 8.6), as suggested by Yavin and Zutra [21], resulted in rapid release of some of these attached vesicles. Increasing the osmolarity of the alkaline buffer greatly increased the yield of vesicles. Examination of the purified vesicle preparation under a phase microscope demonstrated a population of vesicles ranging in diameter from 0.5 to 5.0 μm. As has been demonstrated by other investigators, these vesicles are enriched in plasma membrane when compared to whole cells, have the same sidedness as the membrane of the parental cell, and maintain their lipid asymmetry [21]. The data presented in Table I demonstrate that there is an approx. 5–9-fold enrichment in the plasma membrane marker enzyme, 5′-nucleotidase. The doubling in the cholesterol/phospholipid molar ratio of the plasma membrane vesicles compared to the whole cells (Table I) is consistent with data previously published for vesicles prepared from other cell types [14,15,21]. Thus, the plasma membrane vesiculation observed with the present rat liver cell lines is similar to that reported for other cell types, and the vesicle membranes have the general characteristics of plasma membrane.

Previous studies on cholesterol efflux from cells demonstrated a difference in the rate of cholesterol removal between cell lines [1,2]. This difference is very pronounced between the Fu_5AH hepatoma and the WIRL liver cells [1,2]. Fig. 1 shows a

TABLE I

CHARACTERISTICS OF CELLS AND PLASMA MEMBRANE VESICLES FROM Fu_5AH AND WIRL CELLS

Values are for two independent determinations.

	Fu_5AH		WIRL	
	Cells	Vesicles	Cells	Vesicles
5′-Nucleotidase (μmol P/h per mg protein)	0.83, –	7.74, –	0.23, 0.43	1.35, 2.33
Cholesterol/phospholipid (mol/mol)	0.24, 0.27	0.40, 0.55	0.21, 0.31	0.46, 0.32
Cholesterol/protein (μg/mg)	15, 19	52, 47	12, 14	35, 39
Phospholipid/protein (μg/mg)	109, 120	195, 240	103, 145	156, 250

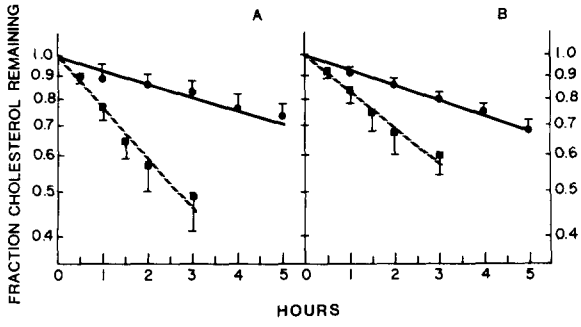

Fig. 1. Efflux at 37 °C of [^{3}H]cholesterol from Fu_5AH rat hepatoma cells and WIRL rat liver cells (A) and plasma membrane vesicles (B) derived from both cell types. The cells or vesicles were exposed to HDL apolipoprotein/egg PC complexes (1 mg PC/ml). ■, Fu_5AH cells or vesicles; ●, WIRL cells or vesicles. Values are means ± S.D. where $n = 6$ for plasma membrane vesicles and $n = 3$ for whole cells.

semi-exponential plot of the fraction of unesterified cholesterol remaining in either whole cells or plasma membrane vesicles (the cholesterol ester content is negligible [2]). The present studies confirm that efflux of at least one-third of the cholesterol in both cell lines follows first-order kinetics with respect to free cholesterol concentration in donor cells when the cells are exposed to HDL apolipoprotein/egg PC complexes, which function as acceptor particles for cellular cholesterol. The kinetics of clearance of free cholesterol from intact cells shown in Fig. 1 are similar to those which have been demonstrated previously with these cells [2]. Exposure of plasma membrane vesilces to the same cholesterol acceptor also gives first-order efflux for at least one-third of the cholesterol (Fig. 1).

Half-times for cholesterol release can be calculated from the experimental data shown in Fig. 1. For Fu_5AH whole cells and plasma membrane vesicles the half-times for cholesterol efflux at 37 °C are 3.2 ± 0.6 h (± S.E., $n = 3$) and 3.9 ± 0.5 h ($n = 6$), respectively. The equivalent times for WIRL cells are 14.3 ± 1.5 h ($n = 3$) and 11.2 ± 0.7 h ($n = 6$). For each cell type, there are no significant differences between the $t_{1/2}$ values for whole cells and plasma membrane vesicles at the $P > 0.05$ level using Student's t-test. These $t_{1/2}$ values clearly demonstrate that the difference in rate of cholesterol release persists in plasma membrane vesicles prepared from the two cell types. Sonication of the plasma membrane vesicle to reduce their diameter from the 0.5–5.0 μm range to approx. 20 nm does not lead to a significant change in $t_{1/2}$ for cholesterol efflux (data not shown). Also, use of formaldehyde and dithiothreitol to induce plasma membrane vesiculation does not influence cholesterol efflux, because $t_{1/2}$ for cholesterol efflux is the same in control cells and in cells which have been exposed to these compounds.

The data presented above demonstrate that the rate of release of cholesterol from cells is a function of the cell plasma membrane. To determine whether the membrane phospholipids play a critical role in determining cholesterol efflux, either total phospholipids from whole cells or the total lipids from plasma membrane vesicles were isolated. It is apparent from the data in Table II that there is no difference in the rate of transfer of [^{14}C]cholesterol from small unilamellar vesicles prepared from plasma membrane total lipids of

TABLE II

CHOLESTEROL TRANSFER FROM SMALL UNILAMELLAR VESICLES FROM Fu_5AH AND WIRL PHOSPHOLIPIDS

There are no significant differences between the $t_{1/2}$ values for WIRL and Fu_5AH lipids at the $P > 0.05$ level using Student's t-test. Results are means ± S.E. n, number of preparations.

Cell line	Half-time (h)			
	Whole cell phospholipid		Plasma membrane lipid	
	26 °C	37 °C	26 °C	37 °C
WIRL	1.0 ± 0.1	0.3 ± 0.1	6.2 ± 0.4	2.9 ± 0.2
	($n = 4$)	($n = 6$)	($n = 19$)	($n = 10$)
Fu_5AH	1.1 ± 0.1	0.5 ± 0.1	5.3 ± 0.3	3.1 ± 0.1
	($n = 4$)	($n = 6$)	($n = 18$)	($n = 6$)

Fu_5AH or WIRL cells. The $t_{1/2}$ values at 26 °C and 37 °C in Table II substituted in the Arrhenius equation give an average activation energy of approx. 18 kcal/mol, which is consistent with previous reports of the temperature dependence of cholesterol efflux from phospholipid vesicles and cells [1,3]. It is interesting that, for both cell types, small unilamellar vesicles prepared from whole cell phospholipid give significantly shorter half-times for [^{14}C]cholesterol transfer than the small unilamellar vesicles prepared from the total lipids from plasma membrane vesicles. This may be due to an increased sphingomyelin content in the latter preparations (see Ref. 14); we have shown that cholesterol desorption from bilayers is slowed as the sphingomyelin/PC ratio is increase (Phillips, M.C. and Rothblat, G.H., unpublished data).

Discussion

Under conditions where desorption from the plasma membrane is rate-limiting for cholesterol efflux, intact cells and isolated plasma membranes give the same $t_{1/2}$ values (Fig. 1). This indicates that the plasma membrane determines the $t_{1/2}$ of the pool of cholesterol we are monitoring. We assume that essentially all cell free cholesterol is located in the plasma membrane (see Refs. 22 and 23) where it behaves as a single kinetic pool for efflux. This implies that cholesterol molecules move to the outer half of the plasma membrane at a rate which is rapid compared to desorption from the plasma membrane. The latter possibility is consistent with recent observations of rapid transmembrane movement ($t_{1/2} < 3$ s) of cholesterol in erythrocytes and fibroblasts [23,24]. The observation that intact cells and isolated plasma membrane vesicles release cholesterol at the same rate implies that intracellular components do not influence efflux of the cell cholesterol mass (greater than 50%) which is in the single kinetic pool with $t_{1/2} \approx 3$ h for Fu_5AH cells (Fig. 1) [2]. The lack of dependence of $t_{1/2}$ for cholesterol efflux on a full complement of cytoplasmic functions indicates that desorption from the plasma membrane does not involve expenditure of metabolic energy in the present cell systems, but rather involves a passive diffusion process. The activation energy of approx. 18 kcal/mol for small unilamellar vesicles formed from membrane and cell phospholipids is associated with the energy required to desorb a cholesterol molecule from the phospholipid bilayer membrane into the aqueous phase, and this is similar for intact plasma membranes [1–3].

Different cell types give different minimum $t_{1/2}$ values for cholesterol efflux to a given acceptor [2], and the maintenance of this difference for efflux from isolated plasma membrane vesicles proves that plasma membrane structure is the governing factor. The location, orientation and molecular motion of cholesterol molecules in biological membranes is not well understood, although the effects of cholesterol molecules on phospholipid molecules have been studied extensively (for a review see Ref. 25). The interactions of cholesterol with its neighboring molecules in the membrane matrix determine the rate at which a cholesterol molecule can desorb into the aqueous phase [3,26]. A quantitative discussion of these effects is not possible at present, but some qualitative inferences can be drawn. Thus, from studies of model and cell membranes systems, the rate of cholesterol exchange or transfer is known to be sensitive to the following parameters.

(1) Degree of unsaturation of phospholipid fatty acyl chains; the $t_{1/2}$ for cholesterol exchange decreases with increasing unsaturation [26–29]

(2) The sphingomyelin/PC ratio; $t_{1/2}$ increases as the sphingomyelin content increases (Phillips, M.C. and Rothblat, G.H., unpublished data).

(3) The PC/cholesterol ratio; in model membrane systems, $t_{1/2}$ decreases somewhat when cholesterol is introduced into the bilayer [26].

Factors 1–3 above describe some potential effects of the lipid composition of membranes on $t_{1/2}$ for cholesterol exchange or transfer, and they may be contributing to the present data for Fu_5AH and WIRL membranes. However, such lipid-mediated effects alone cannot be the cause of the differences in $t_{1/2}$ observed for these two cell types, because small unilamellar vesicles formed from membrane lipids of each cell type give the same $t_{1/2}$ for cholesterol exchange (Table II).

Since the difference in $t_{1/2}$ values for cholesterol efflux from plasma membranes of Fu_5AH and WIRL cells cannot be rationalized in terms of the lipid packing factors (points 1–3 above), other aspects of plasma membrane structure must be

examined for possible explanations. For example, it is possible that cholesterol may desorb at different rates from various plasma membrane regions. The plasma membrane of a hepatocyte is particularly complicated in this respect because, apart from any microdomains of lipids which might exist, the membrane has three well-defined regions: the large sinusoidal region, the contiguous region and the bile canalicular region, which have different transverse distributions of phospholipid [30]. The extent to which each of these regions is retained in these two cultured liver cells is not known. In addition, when small unilamellar vesicles are prepared from the extracted lipids from the membranes, the information about the lateral and transmembrane distribution of the membrane components is lost and compositional averaging occurs. The role of membrane proteins in maintaining the domain structure of hepatocyte membranes and the local organization of cholesterol molecules in these membranes must be elicited before a quantitative understanding of the cholesterol efflux data in Table II can be derived. The presence of a slowly exchanging pool of cholesterol in the rabbit intestine brush-border plasma membrane has been attributed to the interaction of cholesterol with a membrane protein fraction [31].

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References

1 Phillips, M.C., McLean, L.R., Stoudt, G.W. and Rothblat, G.H. (1980) Atherosclerosis 36, 409–422
2 Rothblat, G.H. and Phillips, M.C. (1982) J. Biol. Chem. 257, 4775–4782
3 McLean, L.R. and Phillips, M.C. (1981) Biochemistry 20, 2893–2900
4 Backer, J.M. and Dawidowicz, E.A. (1981) Biochemistry 20, 3805–3810
5 Lund-Katz, S., Hammerschlag, B. and Phillips, M.C. (1982) Biochemistry 21, 2964–2969
6 Bojesen, E. (1982) Nature 299, 276–278
7 Wharton, S.A. and Green, C. (1982) Biochim. Biophys. Acta 711, 398–402
8 Lange, Y., Molinaro, A.L., Chauncey, T.R. and Steck, T.L. (1983) J. Biol. Chem. 258, 6920–6926
9 Slotte, J.P. and Lundberg, B. (1983) Biochim. Biophys. Acta 750, 434–439
10 Pitot, H.C., Peraino, C., Morse, P.A. and Potter, V.R. (1964) Nat. Cancer Inst. Monogr. 13, 229–245
11 Rothblat, G.H., Arbogast, L.Y., Kritchevsky, D. and Naftulin, M. (1976) Lipids 11, 97–108
12 Diamond, L., McFall, R., Tashiro, Y. and Sabatini, D. (1973) Cancer Res. 33, 2627–2636
13 Rothblat, G.H., Arbogast, L.Y., Ouellette, L. and Howard, B.V. (1976) In Vitro 12, 554–557
14 Scott, R.E., Perkins, R.G., Zschunke, M.A., Hoerl, B.J. and Maercklein, P.B. (1979) J. Cell Sci. 35, 229–243
15 Scott, R.E. and Maercklein, P.B. (1979) J. Cell Sci. 35, 245–252
16 Markwell, M.A.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206–210
17 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917
18 Rothblat, G.H. (1974) Lipids 9, 526–535
19 Sokoloff, L. and Rothblat, G.H. (1974) Proc. Soc. Exp. Biol. Med. 146, 1166–1172
20 Widnell, C.C. and Unkeless, J.C. (1968) Proc. Natl. Acad. Sci. USA 61, 1050–1057
21 Yavin, E. and Zutra, A. (1979) Biochim. Biophys. Acta 553, 424–437
22 Amar-Costesec, A., Wibo, M., Thines-Sempoux, D., Beaufay, H. and Berthet, J. (1974) J. Cell Biol. 62, 717–745
23 Lange, Y. and Ramos, B.V. (1983) J. Biol. Chem. 258, 15130–15134
24 Lange, Y., Dolde, J. and Steck, T.L. (1981) J. Biol. Chem. 256, 5321–52323
25 Quinn, P.J. (1981) Prog. Biophys. Mol. Biol. 38, 1–104
26 McLean, L.R. and Phillips, M.C. (1982) Biochemistry 21, 4053–4059
27 Bloj, B. and Zilversmit, D.B. (1977) Biochemistry 16, 3943–3947
28 Poznansky, M.J. and Lange, Y. (1978) Biochim. Biophys. Acta 506, 256–264
29 Nakagawa, Y., Inoue, K. and Nojima, S. (1979) Biochim. Biophys. Acta 553, 307–319
30 Evans, W.H. (1980) Biochim. Biophys. Acta 604, 27–64
31 Bloj, B. and Zilversmit, D.B. (1982) J. Biol. Chem. 257, 7608–7614