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CHOLESTEROL MOVEMENT BETWEEN HUMAN SKIN FIBROBLASTS AND PHOSPHATIDYLCHOLINE VESICLES

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Cholesterol readily exchanges between human skin fibroblasts and unilamellar phospholipid vesicles. Only a fraction of the exchangeable cholesterol and only 10–15% of the total cellular free cholesterol is available for net movement or depletion to cholesterol-free phosphatidylcholine vesicles. [^{14}C]Cholesterol introduced into the fibroblast plasma membrane by exchange from lipid vesicles does not readily equilibrate with fibroblast cholesterol labelled endogenously from [^{3}H]mevalonic acid. While endogenously-synthesized [^{3}H]cholesterol readily becomes incorporated into a pool of esterified cholesterol, little, if any, of the [^{14}C]cholesterol introduced into the fibroblast plasma membrane by exchange from lipid vesicles becomes available for esterification. We interpret these findings as suggesting that: (1) net cholesterol movement from fibroblasts to an acceptor membrane is limited to a small percentage of the plasma membrane cholesterol, and (2) separate pools of cholesterol exist in human skin fibroblasts, one associated with the plasma membrane and the second associated with intracellular membranes, and equilibration of cholesterol between the two pools is a very limited process.

Introduction

The exchange of cholesterol between erythrocytes and either serum lipoproteins or lipid vesicles has been well established [1–4] and has recently been reviewed [5,6]. While a number of authors have identified non-exchangeable pools [7–9], it is now more generally accepted that all of the erythrocyte cholesterol is exchangeable [1,4,5,10–13]. It is important to note that while most of these experiments were performed using isotopic cholesterol under presumed conditions of zero net cholesterol flux, most often determination of net cholesterol movement was not made. For these experiments, lipoproteins and phospholipid vesicles of various lipid composition appear to act in similar fashions (for review, see Ref. 12), dependent primarily on interparticular collisions for cholesterol exchange [13].

Cholesterol movement between lipoproteins and peripheral smooth muscle cells is thought to be an important consideration in the development of peripheral vascular disease [14,41–44]. It has been hypothesized that LDL (low density lipoprotein) is associated with the delivery of cholesterol to peripheral tissue, while HDL (high density lipoprotein) may be associated with the removal of cholesterol from peripheral tissue and its disposal via the liver [15,16]. The net movement of cholesterol between model membrane systems, serum lipoproteins, erythrocytes and lymphocytes has been demonstrated [17–19]. The driving force appears to be the differences in cholesterol-to-phospholipid mole ratios (C/P) in the donor and

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acceptor membranes, with the exception of LDL-dependent, receptor-mediated cholesterol uptake [20,21]. Lange and D'Allesandro [22] demonstrated that for erythrocytes, the depletion of cholesterol (at least the first 40% of membrane cholesterol) and the exchange of [^{3}H]cholesterol appear kinetically indistinguishable. Once the C/P of the erythrocytes has been reduced, both processes, depletion and exchange [23,24], become energetically less favourable. Such a change in the activation energy for the exchange process has been observed [24] and appears to occur at a C/P mole ratio of 0.3. This has been interpreted to represent a change in the packing of the cholesterol and phospholipid molecules as the C/P is reduced and might explain why some authors have observed the existence of multiple pools of cholesterol in erythrocytes and why others have had difficulty in reducing cellular cholesterol below certain C/P values.

Attempts to measure net movement of cholesterol from a variety of cell types, including fibroblasts [25,26], rat hepatoma cells [27], Landschütz ascites cells [28,29] and L5178Y cells [30], have utilized mostly indirect methods. The use of isotopic cholesterol or labelled cholesterol precursors fails to distinguish between exchange and net movement. For measurement of changes in total cellular cholesterol content [29–31] to be valid measurements of cholesterol efflux, it is essential that the metabolic condition of the cell in terms of cholesterol synthesis, the balance between esterified cholesterol and free cholesterol, protein synthesis and cell proliferation be accurately determined.

The role of serum lipoproteins, especially LDL, further complicates the interpretation of net cholesterol movement experiments, since the work of Brown and Goldstein [20] has demonstrated that LDL cholesterol represents the primary source of cellular cholesterol, with internalization of the LDL suppressing endogenous cholesterol synthesis. Goldstein et al. [33] note that free cholesterol from LDL can enter the cell either by endocytosis in association with protein or by a passive exchange reaction between the lipoprotein and the cell membrane. In their experiments, while they demonstrate the uptake of radiolabelled cholesterol from LDL by this latter mechanism, they do not distinguish between net movement and exchange. While it is clear that the receptor-mediated endocytosis delivers cholesterol to the cell interior, it is not clear what the fate of the cholesterol molecule is when it reaches the plasma membrane via the exchange equilibration process. Goldstein et al. [33] have shown that exogenous cholesterol introduced into the cell culture medium in the presence of ethanol and in the absence of serum lipoproteins is able to support cell growth in the presence of compactin, an inhibitor of cholesterol synthesis. Because of its extremely low solubility, it is not possible to determine whether this cholesterol was entering the cell and supporting growth via the passive exchange route or via micropinocytosis of cholesterol crystals or aggregates.

In the current study, we attempt to measure the net flux of cholesterol from human skin fibroblasts to unilamellar lipid vesicles and to compare this process with the isotopic exchange of [^{3}H]-cholesterol between fibroblasts and lipid vesicles. In addition, we attempt (1) to determine whether [^{14}C]cholesterol introduced into the plasma membrane by the exchange process is available for cholesterol esterification within the cell, and (2) to determine the degree of mixing of the two pools of cholesterol, one labelled metabolically via [^{3}H]mevalonic acid and the second labelled with [^{14}C]cholesterol via exchange from lipid vesicles in the absence of serum lipoproteins.

Methods

Human skin fibroblasts. Cultured fibroblasts were derived from foreskins of healthy newborns. Cells were grown in monolayer and used between the 4th and 20th passages. The cells were maintained at 37°C in a humidified incubator containing 5% CO_2. The cells were grown in 75 cm Falcon flasks containing 20 ml of growth medium consisting of Eagle's minimum essential medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), neomycin sulphate (100 μg/ml), 24 mM $NaHCO_3$ and 10% (v/v) fetal calf serum. Media were obtained from Gibco Chemicals (Canada) and sterilized by filtration through a 0.20 μm Nalgene filter. Confluent cells were dissociated with 0.05% trypsin and 0.02% EDTA in Hank's balanced salt solution for 15 min at 37°C.

The cells were plated in fresh flasks at a density of $1 \cdot 10^5$ cells per flask on day 0. Fresh medium was added to the cells on days 2 and 4. For cholesterol exchange and net flux experiments, the cells were used on day 6 before they had reached confluency at a density of $2 \cdot 10^6$ cells per flask. Depending on the experiment, the cells were grown with or without fetal calf serum for the final 24 h prior to initiation of the experiment.

Lipid vesicles. Unilamellar vesicles were prepared from egg phosphatidylcholine with varying amounts of cholesterol. 10 mg of egg phosphatidylcholine (Makor, Jerusalem, Israel) were colyophilized with varying amounts of cholesterol (0, 2.5 and 4.8 mg for cholesterol/phospholipid (C/P) molar ratios of 0, 0.5 and 1.0), dispersed in 10 ml of sterilized Hank's balanced salt solution and sonicated in a Branson W185 sonicator using a standard probe and a power output of 85 watts for 1 h at 4°C under nitrogen using a temperature-controlled cell. The vesicles were centrifuged at $30000 \times g$ for 30 min to remove titanium particles coming off the probe as well as larger multilamellar liposomes. Isotopic tracer studies included 20 μCi of [^{3}H]cholesterol (New England Nuclear) and 5 μCi of [^{14}C]cholesterol oleate (New England Nuclear) added to the phospholipid and cholesterol mixture prior to colyophilization. [^{3}H]Cholesterol was used to determine exchange of cholesterol between vesicles and cells and the [^{14}C]cholesterol oleate was used as a non-exchangeable marker to determine the absorption or uptake of the vesicles by the fibroblasts. Care was taken to avoid contamination of the vesicles as sterilization by filtration was not possible owing to the small size (approx. 250 Å) of the vesicles. Fractionation of vesicles on Sepharose 4B indicated that better than 90% of the lipids and radioactivity eluted as unilamellar vesicles following the void volume.

Cholesterol assay. The assay was used to measure lipid vesicle cholesterol in the medium and both free and esterified cholesterol composition of the fibroblasts or lymphocytes was as described by Gamble et al. [35]. Fibroblasts were prepared for cholesterol assay by dissociation from culture flasks, washing in 12×75 glass centrifuge tubes to remove all growth medium and sonicated for 1 min in the presence of 0.5 ml of 0.1 M potassium buffer, pH 7.4, using the microtip of a Branson W185 sonicaor. Cholesterol composition of the lipid vesicles was determined by first extracting the lipid from the medium (containing Hank's but no serum lipoproteins) by chloroform/methanol extraction followed by solvent evaporation. The assay utilizes cholesterol oxidase to generate H_2O_2, which is subsequently used by a peroxidase to yield a stable fluorescent product from *p*-hydroxyphenylacetic acid. Inclusion of cholesterol esterase in the reaction scheme allows for the determination of cholesterol ester as well as free cholesterol. The assay was carried out as follows: the assay solution was made fresh daily to contain eight parts 0.1 M potassium phosphate buffer (pH 7.4), two parts cholesterol oxidase (1 unit/ml, Boehringer Mannheim), two parts horseradish peroxidase (10 units/ml, Sigma), two parts cholesterol esterase (0.1 unit/ml, Sigma), one part Triton X-100 (0.05%), one part sodium cholate (20 mM) and three parts *p*-hydroxyphenylacetic acid (4 mg/ml). All enzymes were prepared in 0.1 M potassium phosphate buffer (pH 7.4). The cholesterol esterase was omitted in order to determine free unesterified cholesterol. For determination of cholesterol levels in the fibroblast pellet containing 10^5–10^6 cells, 105 μl of a solution containing 20 mM sodium cholate and 1% Triton X-100 were added, followed by 200 μl of 95% ethanol. 1.8 ml of assay solution were added and the glass tube was incubated at 37°C for 60 min, after which fluorescence was measured in a Turner 430 spectrofluorimeter using an excitation bandwidth of 325 nm and an emission bandwidth of 415 nm. Lipid extracts were similarly treated, except that they were dissolved directly in 200 μl of 95% ethanol prior to addition of 2.0 ml assay solution followed by a 30-min incubation. Cholesterol standards between 0.10 and 2.0 μg were treated in the same manner as the lipid extracts. Great care was necessary to utilize only spectral grade solvents, double distilled water, reagent grade chemicals and carefully chromic acid-washed Pyrex glassware.

Results

Fig. 1 demonstrates the linear relationship between cholesterol and arbitrary fluorescence units (previously demonstrated by Gamble et al. [35])

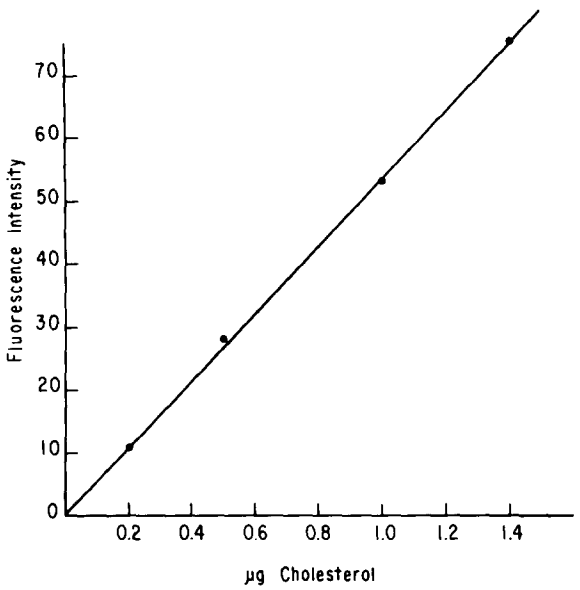

Fig. 1. Standard curve for fluorimetric cholesterol assay [35]. Fluorescence units are arbitrary on the 10× scale of a Turner 430 spectrofluorimeter. If cholesterol esters are to be assayed, then cholesterol esterase is included in the assay medium and the values fall along the same straight line.

for cholesterol values between 100 and 1500 ng. The relationship holds whether the cholesterol is in its free or esterified form when cholesterol esterase is included in the assay solution. The assay was found to be very sensitive to contamination from organic solvents, detergents and various types of disposable glassware. Relatively high concentrations of a number of phospholipids (10 mg/10 ml egg phosphatidylcholine or 5 mg/10 ml sphingomyelin) appeared initially to interfere with the assay, but it was determined that the interference was actually due to cholesterol contamination of these lipids, which are generally purified from sources rich in cholesterol (Poznansky, M.J., Czekanski, S. and Steele, J.A., unpublished data). Contamination represented less than 0.01% of the lipid and no attempts at further purification were attempted. Replicates of the same sample has a standard deviation of less than 5%. Inter-assay variability was less than 10%.

Fig. 2 represents data indicating the ready exchange of [^{3}H]cholesterol between fibroblasts grown on [^{3}H]mevalonic acid and vesicles labelled with [^{14}C]cholesterol. In Fig. 2a, vesicles containing a 0.5:1.0 mole ratio of cholesterol to phospholipid (containing 3 μg free cholesterol) and labelled with [^{14}C]cholesterol were incubated with an excess of confluent human skin fibroblasts (containing 30 μg free cholesterol). Approx. 70%

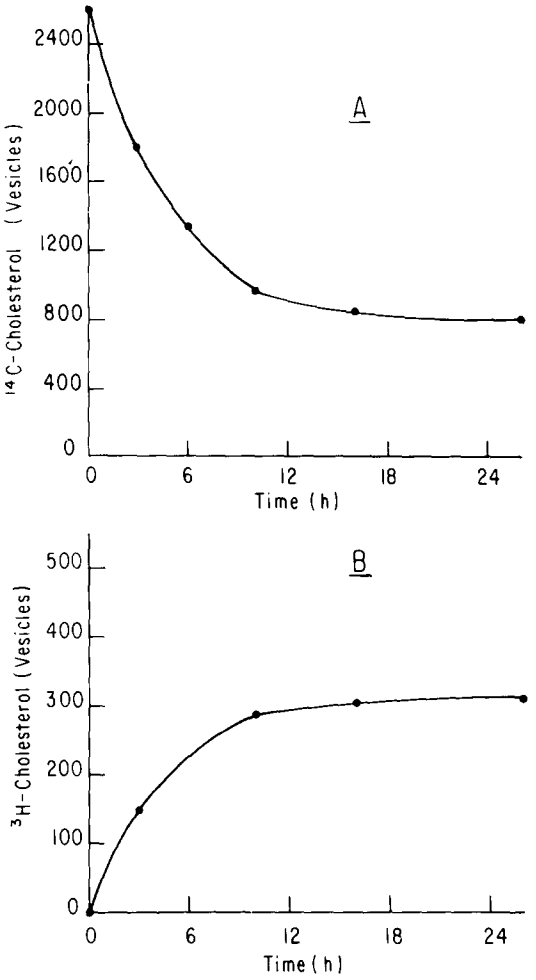

Fig. 2. Exchange of [^{14}C]cholesterol from phospholipid vesicles to human skin fibroblasts (A) and [^{3}H]cholesterol from fibroblasts to vesicles in the medium (B). The acceptor membrane in both cases is present in an approx. 10-fold excess so as to avoid backflux of the radiolabelled cholesterol. The exchange of [^{3}H]cholesterol from confluent fibroblasts (B) to an excess of phospholipid vesicles represents only 25% of the ^{3}H-labelled cholesterol (total) associated with the fibroblasts. C/P ratios of vesicles in both (A) and (B) were 0.5. No net movement of lipids occurred in either experiment. Both figures represent one experiment typical of three. Values for isotope exchange were done in triplicate with values showing standard deviations less than ±5%.

of the vesicle [^{14}C]cholesterol was exchangeable with the fibroblasts and was identified as fibroblast cholesterol (free) following the incubation. No changes in the supernatant cholesterol or phospholipid content could be detected, suggesting that no net movement of lipids was occurring. The kinetics of exchange of [^{3}H]cholesterol from confluent fibroblasts (containing 30 μg free cholesterol) into an excess of lipid vesicles (containing 300 μg free cholesterol) with a C/P ratio of 0.5 seen in Fig. 2b appear similar to the exchange process seen in Fig. 2a. The $t_{1/2}$ for [^{14}C]cholesterol exchange from vesicles to fibroblasts was 3.5 h while the $t_{1/2}$ for [^{3}H]cholesterol exchange from fibroblasts to vesicles was 4.5 h. The [^{3}H]cholesterol that exchanges between the fibroblasts and an excess of lipid vesicles represents only 25% of the fibroblast-free [^{3}H]cholesterol. While the fibroblasts were grown in the presence of fetal calf serum, the experiment was performed in a lipoprotein-free medium. For long-term exchange experiments, human albumin was added to the medium to avoid sticking of vesicles to fibroblasts, but it was shown to have no effect on the exchange process. When vesicles labelled with trace quantities of [^{14}C]cholesterol oleate or [^{3}H]phosphatidylcholine were included in the growth medium at a 10-fold vesicle phosphatidylcholine-to-fibroblast lipid excess, less than 2% of the label was found to bind to the fibroblast, indicating that sticking of vesicles to fibroblasts was not a serious problem. No loss in cell viability could be determined following incubation with lipid vesicles as determined by normal cell shapes and by Trypan blue exclusion.

Fig. 3 represents an attempt to measure the net movement of cholesterol from fibroblasts into cholesterol-free lipid vesicles in the absence of serum lipoproteins. Previous attempts at measuring net movement as opposed to exchange have relied on the measurement of remaining cellular cholesterol after a variety of treatments. This is an indirect measurement and is difficult to assess, since a variety of treatments may alter rates of synthesis of cholesterol, rates of esterification or the degree of hydrolysis of cholesterol esters. To measure the small amounts of net cholesterol flux, it was necessary to utilize the fluorometric assay described in Methods. At 1 h, only 1.6 μg of

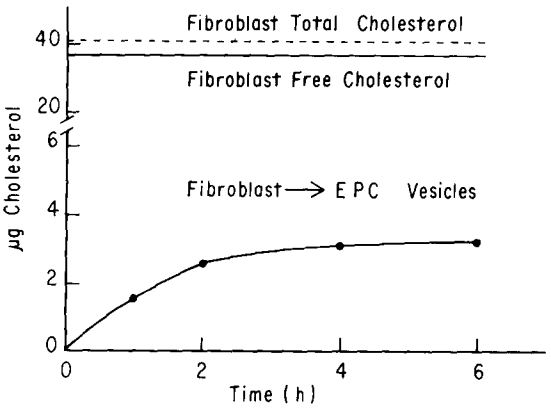

Fig. 3. Net movement of cholesterol between confluent human skin fibroblasts and cholesterol-free phospholipid vesicles. Vesicle phospholipid is present in a 10-fold excess over fibroblast phospholipid. Cholesterol was measured in the lipid extracts from 1-ml aliquots of the suspending medium containing minimum essential medium and 10 mg egg phosphatidylcholine. Addition of fresh lipid vesicles at $t = 12$ h resulted in no additional movement of cholesterol between the fibroblasts and the suspending medium. Addition of trace quantities of [^{14}C]cholesterol oleate or [^{14}C]phosphatidylcholine to the vesicles showed that less than 2% of the vesicles bound to the fibroblast population. No change in fibroblast-free cholesterol (————) or fibroblast total cholesterol (— — —) could be detected. This figure represents one experiment that was typical of five. Cholesterol depletion from fibroblasts to cholesterol-free vesicles ranged from 7% to 14% of the fibroblast cholesterol (free).

cholesterol had moved from the fibroblasts into 10 ml of vesicles. Fig. 3 indicates that only a small proportion of total cell cholesterol could be depleted from the fibroblasts. Fibroblast cholesterol (both free and total) did not change during the course of the experiment. This represents about 12% of the free cholesterol and is approximately half of that amount shown to be exchangeable in Fig. 2b. Three other experiments performed under identical conditions yielded values of 7, 10 and 14%.

Figs. 2 and 3 suggest that only a small fraction of cellular cholesterol (endogenously-synthesized cholesterol) is available for exchange with lipid vesicles in the medium and only a fraction of that cholesterol can be depleted from the fibroblast membrane as seen by the net cholesterol flux measurement. This limited cholesterol depletion by dispersion of cholesterol-free lipids is consistent

with the limits seen for lowering red blood cell cholesterol [23,37]. These authors noted that they were unable to lower red cell C/P values below 0.4 (a value that we must approach in the fibroblasts following incubation with cholesterol-free vesicles). it also suggests that the non-exchangeable pool seen in Fig. 2b might represent intracellular cholesterol that is not available for either exchange or net depletion from the plasma membrane. Such a limitation on the interaction between the plasma membrane and the membrane of intracellular organelles, at least in terms of cholesterol movement, is consistent with and might explain the fact that vastly different C/P values are maintained between these two membranes. In order to test this hypothesis directly, an experiment was devised to label both the plasma membrane and the intracellular cholesterol with two different labels in the same flask of fibroblasts. The scheme of this experiment is described in table I.

[^{3}H]Mevalonic acid was added to non-confluent fibroblasts at the time of passage, or 36 h before the start of the experiment in the case of near-confluent cells. The cells were then washed three times in Dulbecco's phosphate-buffered saline and pulse-labelled for 4 h with [^{14}C]cholesterol from lipid vesicles containing a C/P of 0.5. The cells were again washed and then incubated for 4 h or for 24 h in medium without fetal calf serum. The cells were then exposed for a furter 4 h to non-labelled lipid vesicles with a C/P of 0.5. The vesicles in the supernatant were separated from the cells and the amount of [^{3}H]- and [^{14}C]cholesterol and cholesterol esters in both fibroblasts and vesicles were determined following separation of lipids on TLC plates.

Tables I and II represent the results of these experiments presented in terms of ratios of ^{3}H to ^{14}C or ratios of metabolically-labelled cholesterol to cholesterol introduced into the fibroblasts by exchange from vesicles. The ^{3}H/^{14}C ratio in the vesicles is assumed to represent a sampling of the cholesterol composition of the fibroblast plasma membrane. The ratio is always lower in the vesicles than it is in the fibroblast lipid extract. Furthermore, the ratio does not change much if an additional 20 h is given for the various cholesterol pools to equilibrate. The fibroblast lipid extract was further fractioned on TLC to determine ^{3}H and ^{14}C in both free and esterified cholesterol. Virtually no ^{14}C label was found in the cholesterol ester spot, giving rise to very high ^{3}H/^{14}C ratios. By using vesicles with C/P mole ratios of 0.5, approximating that of the fibroblast plasma membrane, we were able to show (as in the experiments in Figs. 2a and b) that no net cholesterol movement from fibroblasts to vesicles occurred in these incubations.

TABLE I

EXCHANGE OF [^{3}H]CHOLESTEROL FROM FIBROBLASTS AND [^{14}C]CHOLESTEROL FROM PHOSPHOLIPID VESICLES

Human skin fibroblasts were grown for 72 h in the presence of [^{3}H]mevalonic acid. Then at $t=0$, the fibroblasts were washed and pulse-labelled with [^{14}C]cholesterol from phospholipid vesicles containing a cholesterol/phospholipid ratio of 0.5 for a 4-h period. The cells were again washed and incubated for a further 4 h or 24 h in medium containing lipoprotein-deficient serum. Unlabelled phospholipid vesicles (C/P=0.5) were then added to the incubation medium, and following a further 4-h incubation, the cells were separated from the vesicles in the supernatant and the ratio of [^{3}H]cholesterol to [^{14}C]cholesterol determined in both the fibroblasts and vesicles. Expt. 1 was performed using confluent cells, while Expt. 2 was performed on non-confluent cells.

	[^{3}H]Cholesterol/[^{14}C]Cholesterol	
	Expt. 1	Expt. 2
Vesicles (4 h)	0.198	0.208
Fibroblasts (4 h)	0.678	0.458
Vesicles (24 h)	0.248	0.250
Fibroblasts (24 h)	0.599	0.433
Fibroblasts (24 h) [a]		
Cholesterol	0.551	0.378
Cholesterol esters	40.0 [b]	25.5 [b]

[a] Lipids were extracted from the fibroblasts and cholesterol esters and cholesterol were separated by thin-layer chromatography. No cholesterol ester was present in the vesicle fraction.

[b] Less than 20 dpm ^{14}C in ester spot.

Four conclusions can be drawn from these experiments: (1) [^{14}C]Cholesterol introduced into the plasma membrane by exchange from phospholipid vesicles does not readily equilibrate with cellular metabolically-labelled [^{3}H]cholesterol. (2) Between 4 and 24 h, there is little change in the ^{3}H/^{14}C ratio of cholesterol available for exchange to

TABLE II

ESTERIFICATION OF [^{14}C]CHOLESTEROL INTRODUCED INTO FIBROBLASTS FROM PHOSPHOLIPID VESICLES

Growth conditions and labelling conditions were as described in the text and in Table I, except that the incubation medium contained 10% fetal calf serum in the 24-h period between incubations with radioactively labelled and non-labelled vesicles. The lipoprotein-rich serum was included in order to avoid inhibiting the mechanism of cholesterol esterification.

	[^{3}H]Cholesterol/[^{14}C]Cholesterol
Vesicles (0 h)	0.395
Fibroblasts (0 h)	1.40
Vesicles (24 h)	0.448
Fibroblasts (24 h)	1.270
Fibroblast extract [a]	
Cholesterol	1.175
Cholesterol esters	20.0

[a] Raw counts of cholesterol and cholesterol esters in the fibroblast extract include [^{3}H]cholesterol 850 dpm, [^{14}C]cholesterol 723 dpm, [^{3}H]cholesterol esters 98 dpm, and [^{14}C]cholesterol esters 5 dpm.

vesicles from the plasma membrane, suggesting very little additional exchange of cholesterol between the intracellular regions and the plasma membrane during that time period. (3) The non-exchangeable pool of [^{3}H]cholesterol is not accounted for by [^{3}H]cholesterol ester since the different ratios between the vesicles and fibroblasts is retained if only fibroblast cholesterol (free) is considered. (4) Virtually no [^{14}C]cholesterol exchanged into the fibroblast from the first vesicle incubation is esterified to form [^{14}C]cholesterol ester within the cell during the period of incubation.

The fact that none of the [^{14}C]cholesterol exchanged into the fibroblasts from the vesicles was esterified was not entirely surprising, since it could be explained by the fact that this step is inhibited in the absence of LDL [20]. We therefore carried out the second incubation (24 h in the absence of vesicles) in the presence of fetal calf serum. Under these conditions, it would not be expected that the process would be inhibited, and yet the extremely low incorporation of exchanged [^{14}C]cholesterol into cholesterol esters is maintained (Table II).

Discussion

Experiments with red blood cells and model membrane systems have suggested that cholesterol depletion and loading depends primarily on the cholesterol-to-phospholipid ratio in the donor and acceptor membrane (or lipoprotein) [17]. The simplicity of many of these experiments led to the suggestion that cholesterol moves easily between various compartments. Two points suggest that such an interpretation may be incorrect for more complex cellular systems. Most of the cholesterol flux experiments utilize isotopic flux measurements and fail to distinguish between cholesterol exchange and net cholesterol movement. While it has been suggested [22] that the process of cholesterol exchange from red cells cannot be kinetically distinguished from net cholesterol depletion, this may only be the case for cells with C/P ratios close to unity. Other authors [12,13] have shown that there is a limit to the degree of cholesterol depletion that can be produced in red cells. A second question that suggests that equilibration of cholesterol between membranes may be too simple an explanation for cholesterol movement between membranes is the maintenance of large differences in C/P ratios between plasma membranes and the membranes of intracellular organelles [12,34].

An understanding of the pathways for cholesterol movement may explain the mechanism by which low intracellular organelle cholesterol is maintained in the face of high plasma membrane levels. It might also define the importance of the passive equilibration of cholesterol between lipoproteins and plasma membranes and the role of this pathway in the control of cholesterol biosynthesis. The control of net cholesterol movement may also be important in understanding how HDL may lower cholesterol levels in peripheral tissue by carrying cholesterol to the liver for disposal. Stein et al. [32,39] demonstrated net cholesterol flux from cells grown in tissue culture into plasma lipoproteins with and without added phospholipids, but in all cases cholesterol levels were measured in the cells and not in the media into which the cholesterol was being depleted. Other authors have claimed demonstration of cholesterol efflux from cultured cells, but in most cases [25,27,28],

isotopic cholesterol movement was being monitored. Phillips et al. [31] also observed a rather limited cholesterol removal from cultured fibroblasts, although they monitored changes in cell cholesterol rather than appearance of cholesterol in the medium.

The present results indicate that net movement of cholesterol between human skin fibroblasts and lipid vesicles is far more limiting than the net movement of cholesterol between human erythrocytes and either lipid vesicles or lipoproteins. In the first instance, it appears that only a fraction of the fibroblast cholesterol is exchangeable, supposedly that fraction associated with the plasma membrane. Secondly, only a fraction of the exchangeable cholesterol can be depleted from the fibroblasts, suggesting that the plasma membrane could not draw upon intracellular cholesterol pools when the C/P ratio was lowered. An experiment designed to determine whether cholesterol introduced by exchange to the plasma membrane became available or mixed with intracellular pools of cholesterol showed that cholesterol synthesized and labelled with ^{3}H endogenously undergoes little mixing with [^{14}C]cholesterol introduced into the plasma membrane by exchange from lipid vesicles. Furthermore, none of the [^{14}C]cholesterol introduced into the cell by the exchange mechanisms becomes available for esterification, even under conditions where cholesterol ester formation is not inhibited by the absence of LDL. These results are in agreement with others [27] who demonstrated that cholesterol from cholesterol-rich lipid dispersions could accumulate as cholesterol esters in Fu5AH rat hepatoma cells only if serum lipoproteins were present in the incubation medium.

These results suggest a number of points: (1) Simple exchange processes and depletion to lipid vesicles appear not to be an effective method for lowering cholesterol levels in peripheral cells such as fibroblasts. (2) Isotopic exchange of cholesterol between fibroblasts and lipid vesicles is not indicative of net cholesterol efflux. (3) Cholesterol introduced into the plasma membrane by exchange from vesicles does not readily equilibrate with intracellular pools of cholesterol. This last point may partially explain the ability of intracellular membranes and plasma membranes to retain vastly different C/P ratios. The manner in which the plasma membrane attains high levels of cholesterol is unknown. It is possible to speculate that it is related to high levels of sphingomyelin found in the plasma membrane of many cells; this particular phospholipid being absent in the membrane of intracellular organelles and supposedly having a high affinity for cholesterol [40]. Another possibility that has not been adequately investigated is that plasma membrane cholesterol may be derived from and in equilibration only with plasma lipoproteins, thereby explaining the large C/P differential between the intracellular membranes and the plasma membrane.

Acknowledgements

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