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Serum albumin enhances the uptake of [^{3}H]cholesterol from phosphatidylcholine vesicles by cultured human fibroblasts

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Cultured human lung fibroblasts, incubated with cholesterol/phosphatidylcholine vesicles (cholesterol:phosphatidylcholine molar ratio 1:1) incorporated vesicle [^{3}H]-cholesterol linearly for at least 48h by an exchange process without gaining sterol mass. The incorporation of [^{3}H]cholesterol by the cells was markedly enhanced in the presence of purified bovine serum albumin. A fraction of the incorporated vesicle [^{3}H]cholesterol was esterified by the cells.

The exchange of unesterified cholesterol between different biological lipid structures, e.g. cell membranes and lipoproteins, is a spontaneously occurring process both *in vitro* and *in vivo* (for a review, see Bruckdorfer & Graham, 1976). The molecular mechanism behind the exchange process is probably closely related to the fact that all amphiphilic lipids undergo monomer–aggregate equilibria in a water phase (Tanford, 1980). Results obtained by Phillips and co-workers (Phillips *et al.*, 1980; McLean & Phillips, 1981; Rothblat & Phillips, 1982; see also Wharton & Green, 1982) have indicated that unesterified cholesterol moves from one lipid compartment into another by molecular diffusion through the water interphase.

Although the lipid metabolism of cultured cells has been the subject of intense investigations during the last decades, very little is known about the metabolism of exchangeable cholesterol in cells. There is one report which indicates that cultured human fibroblasts do not esterify exchangeable cholesterol and thus cannot use plasma membrane-derived cholesterol for intracellular metabolism (Poznansky & Czekanski, 1982). We have, however, recently reported some findings which show that at least some cell-lines of human fibroblasts and rat smooth muscle cells were able to esterify exogenous cholesterol that was incorporated from lipid vesicles by exchange (Slotte *et al.*, 1984; Slotte & Lundberg, 1983). The present work is focused on the movement of unesterified cholesterol between cholesterol/phosphatidylcholine vesicles (1:1 molar ratio) and cultured human lung fibroblasts during long-term incubations. We have also determined the effects of serum albumin on the exchange of cholesterol and measured the rate of esterification of exogenous vesicle-derived cholesterol.

Materials and methods

Reagents

[1,2(n)-^{3}H]Cholesterol (47Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Reagent grade cholesterol was purchased from Fluka AG, Buchs, Switzerland. Chromatographically purified egg phosphatidylcholine, bovine serum albumin, and reagents for cholesterol mass determinations were obtained from Sigma (St. Louis, MO, U.S.A.). Sepharose 4B was from Pharmacia, Uppsala, Sweden.

Cell culture

Human non-transformed and diploid lung fibroblasts (Li 83), obtained from the State Bacteriological Laboratory in Stockholm, were maintained as cell monolayers in Falcon plastic tissue culture flasks (75cm^2) in a mixture of William's E-medium and Ham's F-12 (1:1, v/v) (Gibco Europe) supplemented with 5% (v/v) foetal-calf serum and 50μg of neomycin/ml. The cells were used between the 10th and the 20th passage.

Lipid vesicles

Lipid vesicles containing a 1:1 molar ratio of [^{3}H]cholesterol (4700c.p.m./μg) and egg phosphatidylcholine (with 0.1% of butylated hydroxytoluene as antioxidant) were prepared by co-lyophilization and subsequent ultrasonic irradiation in phosphate-buffered saline (pH7.4) at a cholesterol concentration of 1mg/ml, as described elsewhere (Slotte *et al.*, 1984).

Delipidized albumin

Bovine serum albumin was delipidized according to the method reported by Rothblat *et al.* (1976). Before the serum albumin was used for incubation studies, the preparation was purified by column chromatography on Sepharose 4B (eluted with 0.05M-Tris/HCl in 0.15M-NaCl, pH7.4) and concentrated with a Minicon concentrator (Amicon) to about 200–300mg/ml. The concentrate was sterilized by filtration (0.45μm pore size).

Incubation procedures

All experiments were performed with near-confluent cells in 60mm diameter Petri dishes (about 400μg of cell protein/dish). Before the incubations were started the complete growth medium was replaced by a serum-free medium (Ham's F-12). Purified and delipidized serum albumin and vesicle [^{3}H]cholesterol were added to the dishes in small volumes and the total incubation volume was adjusted to 3ml. After completed incubations the cell monolayers were washed three times with phosphate-buffered saline and detached by gentle scraping in 1.0ml of 0.05% sodium dodecyl sulphate solution. Aliquots of the cell suspension were taken for protein determination (100μl), total radioactivity (100μl), and for lipid extraction (800μl).

Analytical procedures

Cellular cholesterol and cholesteryl ester contents were determined by the enzymic method of Gamble *et al.* (1978). Free and esterified labelled cholesterol was extracted with chloroform/methanol (2:1, v/v) containing lipid carriers. The neutral lipids were separated on t.l.c. plates (DC-Alufolien Kiselgel 60; Merck, Darmstadt, Germany) with n-hexane/diethyl ether (1:1, v/v) as solvent. The distribution of lipid radioactivity between free and esterified cholesterol positions was determined by cutting the respective regions into liquid-scintillation vials. Insta-Fluor (Packard) scintillation cocktail was added 60min before counting. Cell protein was determined by the modified Lowry method (Markwell *et al.*, 1978).

Results and discussion

The surface transfer of free cholesterol was studied in a model system with lipid vesicles (cholesterol:phosphatidylcholine 1:1) and cultured human lung fibroblasts. In this experimental design all of the [^{3}H]cholesterol was initially in the lipid vesicle pool. Since there will most certainly be a partial back transfer of labelled tracer from cells into vesicles, at least during prolonged incubations, relative units of the transferred [^{3}H]-cholesterol have been given in the Figures.

Studies by Mayhew *et al.* (1980) and Poznansky & Czekanski (1982) have shown that the interaction between cholesterol/phosphatidylcholine vesicles and cultured fibroblasts does not result in any significant vesicle–cell fusion or vesicle endocytosis by the cells. In our experiments about 3% of the total vesicle load was adsorbed to the cells (per mg of cell protein) during a 5h incubation at 4°C in a serum-free incubation medium. This unspecific adsorption of vesicles to cells has been corrected for in the cholesterol exchange results.

As shown in Fig. 1, fibroblasts incorporated [^{3}H]cholesterol from the vesicles linearly for at least 48h in the absence of serum proteins. This shows that no isotopic equilibrium was achieved during this period. Based on the specific radioactivity of the vesicle [^{3}H]cholesterol it can be calculated that after 48h about 9μg of vesicle [^{3}H]-cholesterol had been exchanged with the cellular free sterol. This amount corresponds to about 28% of the cellular free sterol. These calculations do not, however, correct for any back-transfer of isotopic label from cells to vesicles. The incorporation of vesicle [^{3}H]cholesterol did not lead to any major increase of the cellular sterol mass (Table 1), indicating that a roughly one-for-one molecular exchange was taking place between vesicles and cell membranes. This one-for-one molecular exchange is explained by the almost identical

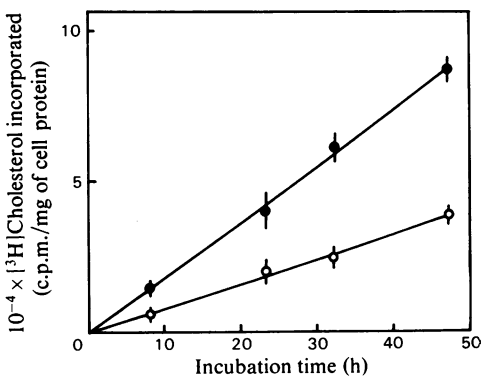

Fig. 1. *Incorporation of vesicle [^{3}H]cholesterol by cultured human lung fibroblasts*

Near-confluent cells in 60mm Petri dishes (about 400μg of cell protein) were incubated with 50μg of vesicle [^{3}H]cholesterol (cholesterol:phosphatidylcholine molar ratio 1:1) in a serum-free incubation medium with 10mg of purified bovine serum albumin/ml present (●) or without albumin (○). The radioactivity has been corrected for the unspecific binding of vesicles to cells at 4°C. The incubations were performed as described in the Materials and methods section. The values are the mean of triplicate determinations ±S.D.

cholesterol/phospholipid molar ratios of the two lipid compartments (1:1 in vesicles and 0.8–0.9:1 in plasma membranes; Riordan *et al.*, 1979). The slightly higher cholesterol mass in cells incubated with vesicles compared with control cells (Table 1) may be due to the unspecific binding of vesicles to cells, which has not been corrected for in the quantitative determinations.

Although the vesicle [3H]cholesterol was transferred to cells readily in the absence of serum proteins, purified and delipidized bovine serum albumin markedly increased the incorporation of [3H]cholesterol into the cells (Fig. 1). During the 48h incubation about 60% of the cellular free cholesterol was exchanged with vesicle [3H]-cholesterol in the presence of serum albumin. This effect of serum albumin on the cholesterol-exchange process has been observed previously (Rottem *et al.*, 1981; Tyrrell *et al.*, 1977; Slotte & Lundberg, 1983). The underlying mechanism behind this effect is not known. The effect of albumin on the rate of vesicle [3H]cholesterol incorporation into cells was not, however, due to an induced vesicle–cell fusion since cells incubated with albumin and vesicles for 24h did not contain significantly more sterol mass than did cells incubated with vesicles only (Table 1).

In previous short-term incubations with this cell type the formation of [3H]cholesteryl esters was detectable after a lag of about 45min (Slotte *et al.*, 1984). During long-term incubations the fibroblasts esterified exchangeable exogenous [3H]-cholesterol almost linearly for at least 48h (Fig. 2). The amount of vesicle-derived [3H]cholesterol that was esterified by the cells correlated rather well with the amount of [3H]cholesterol that was incorporated into the plasma membranes. More [3H]cholesteryl esters were formed when the [3H]-cholesterol incorporation was enhanced by serum albumin, but the ratio between [3H]cholesteryl esters formed and the total amount of label incorporated was not markedly affected by the presence of purified albumin. After 48h about 14% of the incorporated [3H]cholesterol was esterified with albumin present, whereas about 12% was esterified in a protein free incubation.

These results would indicate that a fraction of the plasma membrane cholesterol is in a dynamic balance both with extracellular free cholesterol (vesicles) and with a fraction of the intracellular free cholesterol which is the substrate for esterifying enzymes. Since an enhanced rate of [3H]-cholesterol incorporation led to an increased formation of [3H]cholesteryl esters, one can conclude that the intracellular esterification of exchangeable cholesterol was not the rate-limiting step in the process. Rather the supply of [3H]-cholesterol to the cellular plasma membranes (i.e. the exchange rate) was limiting the formation of [3H]cholesteryl esters in these cells.

The biological significance of these findings is that extracellular free cholesterol may serve as a substrate for intracellular esterifying enzymes, provided that the extracellular free cholesterol is incorporated into the cells (e.g. by transfer into the plasma membranes). The quantitative contribution of this exchange process to the formation of cellular steryl esters, as related to the uptake and esterification of low-density-lipoprotein cholesterol, remains to be determined by a more quantitative approach.

Table 1. *Cholesterol content of cultured human lung fibroblasts after incubation with cholesterol:phosphatidylcholine vesicles (molar ratio 1:1) with or without purified serum albumin*

The cells were incubated in 60mm diameter Petri dishes at 37°C with 100μg of vesicle [3H]cholesterol per dish for 24h. Bovine serum albumin was added to give a final concentration of 10mg/ml. Control cells were incubated in serum-free Ham's F-12 medium. Values are means ± S.D. ($n = 4$).

Incubation conditions	Free cholesterol (μg/mg)	Esterified cholesterol (μg/mg)
Control cells	31.4 ± 1.2	4.2 ± 1.3
Cells incubated with vesicles	34.4 ± 0.9	7.2 ± 3.0
Cells incubated with vesicles + albumin	33.4 ± 1.6	6.2 ± 0.8

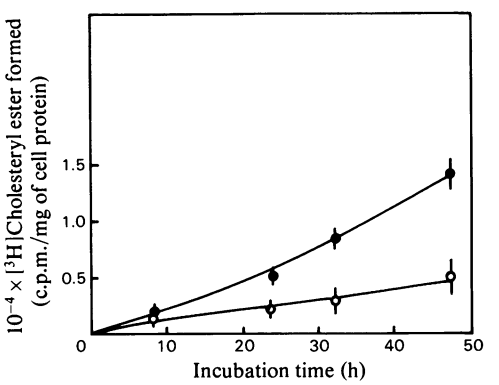

Fig. 2. *Esterification of exchangeable vesicle [3H]-cholesterol by the cells*

The cells (about 400μg of cell protein) were incubated with 50μg of vesicle [3H]cholesterol with (●) or without (○) albumin present (10mg/ml) in a serum-free incubation medium. The amount of [3H]-cholesterol esterified by the cells was determined as described in the Materials and methods section. Each point is the mean of triplicate determinations ± S.D.

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