# CHOLESTEROL EXCHANGE BETWEEN PHOSPHOLIPID VESICLES

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*Abstract*: The mechanism of cholesterol exchange has been investigated by following the transfer of radiolabeled cholesterol from negatively charged, unilamellar donor vesicles to neutral acceptor vesicles. Donor and acceptor vesicles were incubated together and were stable to fusion over the course of the experiment. At intervals, donor and acceptor vesicles were separated by passage through a column of DEAE-Sepharose. Ion exchange chromatography was used for rapid separation of donor and acceptor (present in 10-fold molar excess) vesicles. The kinetics of [$^{14}$C] cholesterol transfer between unilamellar vesicles was described and analyzed in the presence of sucrose buffer; 10% FCS (fetal calf serum) and 50% FCS. The process is first order kinetics. This means that acceleration of the off-rate must be due to donor-acceptor interactions at short distances. It was found that the presence of serum in the medium greatly diminished rather than increased the total transfer of radiolabelled cholesterol from donor vesicles to the acceptor vesicles.

*Key words*: cholesterol exchange, serum, radiolabel, chromatography, liposomes.

## INTRODUCTION

An important aspect of cellular lipid metabolism is the exchange and transfer of cholesterol between cell membranes and lipoproteins [13]. It is important to understand this exchange process which affects the efficiency of drug (gene) delivery and the choice of liposome components in the design of liposomes as drug (gene) carriers.

However, numerous complications arise in the investigation of lipid exchange in biological systems due to protein and lipid transfer, adsorption of vesicles to membranes, and the complex structures of biological membranes and lipoproteins [5, 9]. In an appropriate model system, these complications may be minimized so that in the absence of fusion, one of two limiting mechanisms may operate in lipid exchange: (1) lipid molecules diffuse through a complex formed by the transient fusion of two lipid monolayers or

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bilayers following collision of the two particles ("collision complex"; [6]) or (2) free lipid molecules diffuse through the aqueous phase separating the donor and acceptor particles [13].

The vast majority of studies of this process have demonstrated that the rate of transfer is independent of vesicle concentration under the usual experimental conditions. This result suggests that the rate-limiting step in transfer is lipid desorption from the bilayer with subsequent rapid diffusion through the aqueous phase. This transfer mechanism has been supported by numerous studies on phospholipids transfer [14, 19], as well as in studies concerning transfer of cholesterol [4] and sphingolipids [1, 2], while a much more limited number of studies support a mechanism by which lipids transfer upon vesicle collisions [10, 12].

Transfer is typically determined by one of two general methods. In one of them, movement of a fluorescent phospholipid derivative [13, 14, 19, 20] or of a radiolabeled phospholipid analogue [3, 17] between vesicle populations is monitored.

Liposomes have been and are being used to study the inter-membrane transfer rates of natural membrane components like cholesterol. Depending on the size of the liposomes and lipid composition and the properties of the drug, the spontaneous transfer half-life may be in the range from milliseconds to several days.

Liposomes of sufficiently different surface charge made possible the rapid separation of these liposomes on an ion-exchange column. Small unilamellar vesicles of pure phospholipid and cholesterol have been used a great deal in the study of the exchange of these molecules because well defined donor and acceptor particles can be formed.

Under appropriate incubation conditions, the small unilamellar vesicles are stable to aggregation and fusion so that interpretation of the kinetic data is not complicated by consideration of binding of donor and acceptor particles. Also, there is not the possibility of any proteins perturbing the lipid exchange process [18].

In this study, the kinetics of [$^{14}$C] cholesterol transfer between unilamellar vesicles was described and analyzed in the presence of sucrose buffer; 10% FCS and 50% FCS. Studies in model systems have established that free cholesterol molecules can transfer between membranes by diffusion through the intervening aqueous layer. Desorption of free cholesterol molecules from the donor lipid-water interface is rate-limiting for the overall transfer process and the rate of this step is influenced by interactions of free cholesterol molecules with neighboring phospholipid molecules.

## MATERIALS AND METHODS

### MATERIALS

1-Palmitoyl-2-oleoyl-3-sn-Glycerophosphocholine (POPC) was obtained from (Genzyme, Switzerland), egg phosphatidylcholine (EiPC) from Lipoid. Sodium azide, Trizma pre-set crystals and dicetylphosphate (DCP) were purchased from Sigma, Mo, USA.

Tris ultra pure buffer and sodium deoxycholate were obtained from (ICN Biomedicals Inc.). Diethylaminoethyl (DEAE) Sepharose CL-6B was purchased from Pharmcia Biotech (Sweden). Sucrose was obtained from Merck. Cholesterol (CHOL) was obtained from Calbiochem (CA, USA). All other reagents were of analytical reagent grade.

### PREPARATION OF LIPOSOMES

Liposomes were formulated according to well-established methods of extrusion [16]. In short: the appropriate phospholipids composition was mixed in organic solvent in a 50 ml round flask. The organic solvent was evaporated to dryness by a rotary evaporator. The resulting thin film was hydrated with tris buffer saline (10 mM tris, 140 mM NaCl, and pH 7.4). The resulting lipid suspension was extruded through 100 nm polycarbonate membranes, using a commercially available extruder (Liposofast, Avestin Inc., Canada). The donor vesicles composed of egg phosphatidylcholine (EiPC); decityl (DCP) and (CHOL) in molar ratio (7:1:2) respectively, with 1 µCi/ml [$^{14}$C] CHOL and POPC: CHOL (8:2) vesicles were used as acceptors.

### CHARGED VESICLE (TRANSFER) ASSAY

[$^{14}$C] cholesterol transfer was monitored with a charged vesicle assay which is based on the method originally described by Hellings *et al*. [7]. This assay employs two vesicle populations, negatively charged donor and neutral acceptor vesicles. Lipid transfer was determined by analyzing the movement of label from the donor to the acceptor fraction as a function of time. A given concentration of donor and acceptor vesicles was mixed in a relation of (1:10) in the presence of sucrose buffer; 10% FCS and 50% FCS at 25°C.

At appropriate time intervals, a 0.01 ml aliquot was placed on a mini-column containing 0.5 ml of DEAE-Sepharose CL-6B (that selectively retains the donor by virtue of its charge) which had been pre-equilibrated with 0.02 ml of acceptor vesicles to reduce nonspecific adsorption and improve recovery of acceptor vesicles. The neutral acceptor vesicles were recovered by elution with 1.5 ml of iso-osmolar sucrose buffer (290 mM sucrose, 10 mM Trizma

pre-set crystals and 0.02% Na azide, pH 7.4). This eluate was collected directly into liquid scintillation vials (for radiolabeled cholesterol) and the samples were analyzed by liquid scintillation counting of $^{14}C$. The same experiments were done with incubation of the donor vesicles only with 10% FCS and 50% FCS.

## RESULTS AND DISCUSSION

An *in vitro* system for measuring the transfer of cholesterol molecules from liposomal components to model membranes mimicking other membranous binding places in the body (erythrocyte membranes, endothelial cell membranes, LDL, etc.) was developed, analogous to the assay proposed by McLean and Philips (13).

Liposomes have been and are being used to study the inter-membrane transfer rates of natural membrane components like cholesterol. The inter-membrane transfer phenomenon is first described as part of membrane biochemistry studies with liposomes as model membranes for biological membranes.

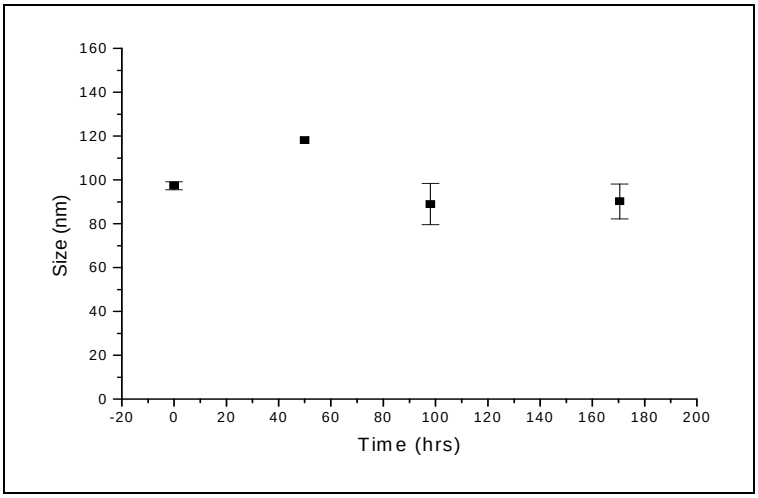


Fig. 1. Liposome size measurements with time during the transfer experiment. Donor liposomes (EiPC/DCP/CHOL (7:1:2)) were loaded with $^{14}C$ Chol and mixed with acceptor neutral liposomes (POPC/CHOL (8:2) in a relation (1:10) at 25 °C. In the presence of iso-osmolar sucrose buffer (290 mM sucrose, 10 mM Trizma pre-set crystals and 0.02% Na azide, pH 7.4).

Under appropriate incubation conditions, the small unilamellar vesicles were stable to aggregation and fusion so that interpretation of the kinetic data is not complicated by consideration of binding of donor and acceptor particles. The size of donor and acceptor vesicles was stable (100 nm ± 10) during the incubation time (Fig. 1) which agrees with that reported before [18].

The transfer of label from donor vesicles to acceptor vesicles was monitored with time in the presence of sucrose buffer; 10% FCS and 50% FCS. The data obtained from incubation performed at 25 $^{o}$C were fitted to exponential decay functions.

The transfer of radio labeled lipid from the donor to the acceptor vesicles was monitored with time in presence of sucrose buffer (Fig. 2). There was an initial burst of transfer during the first few minutes of reaction. This rapid phase was followed by apparent first order kinetics with a half-time of a few hours, as commonly reported [21]. The half life time for cholesterol transfer was 4.04 hrs. This observation of first-order kinetics has been generally accepted to indicate that lipid transfer occurs by way of rate-limiting monomer desorption from donor vesicles followed by rapid diffusion through the aqueous phase to acceptor vesicles [11]. This means that acceleration of the off-rate must be due to donor-acceptor interactions at short distances.

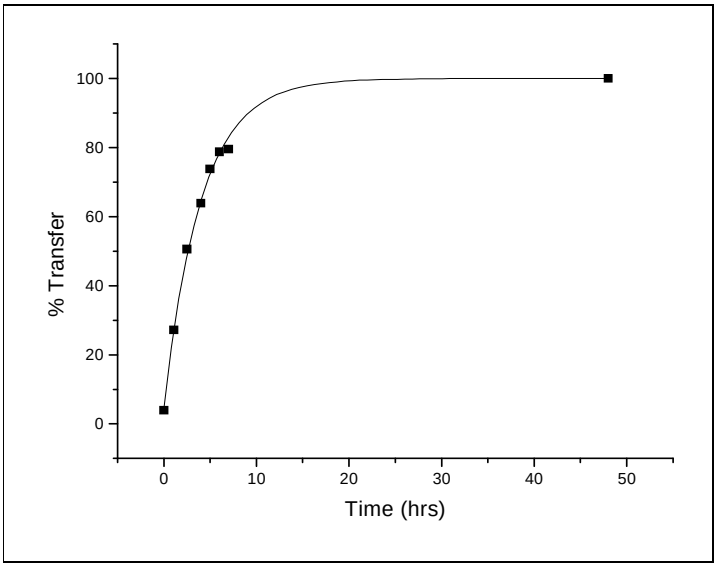


Fig. 2. Percent transfer of $^{14}$C-Chol between liposomal membranes in iso-osmolar sucrose buffer. For more details, see under Figure 1.

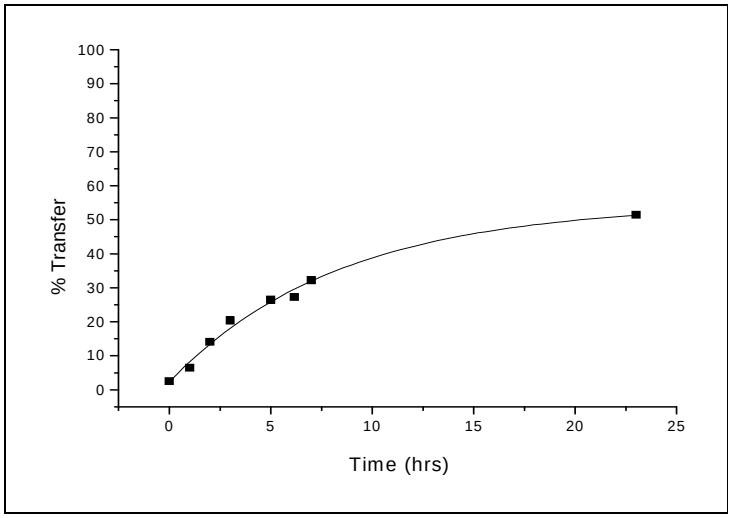


Fig. 3. Percent transfer of $^{14}$C-Chol between liposomal membranes in the presence of 10% FCS. For more details, see under Figure 1.

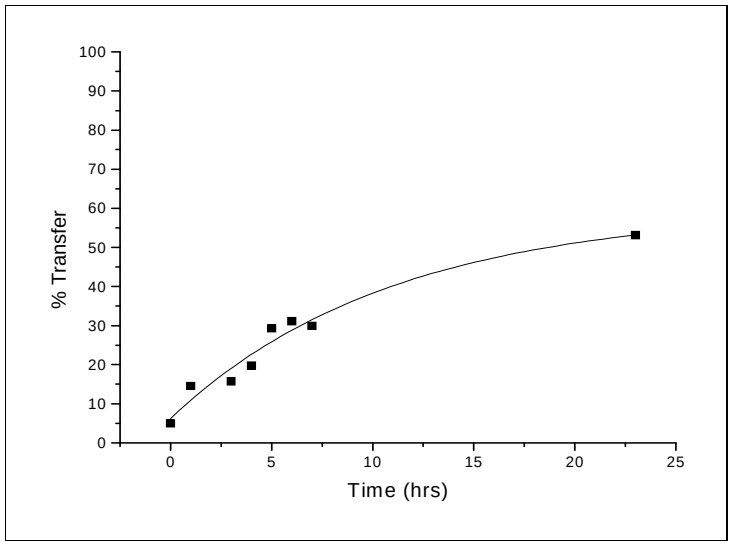


Fig. 4. Percent transfer of $^{14}$C-Chol between liposomal membranes in the presence of 50% FCS. For more details, see under Figure 1.

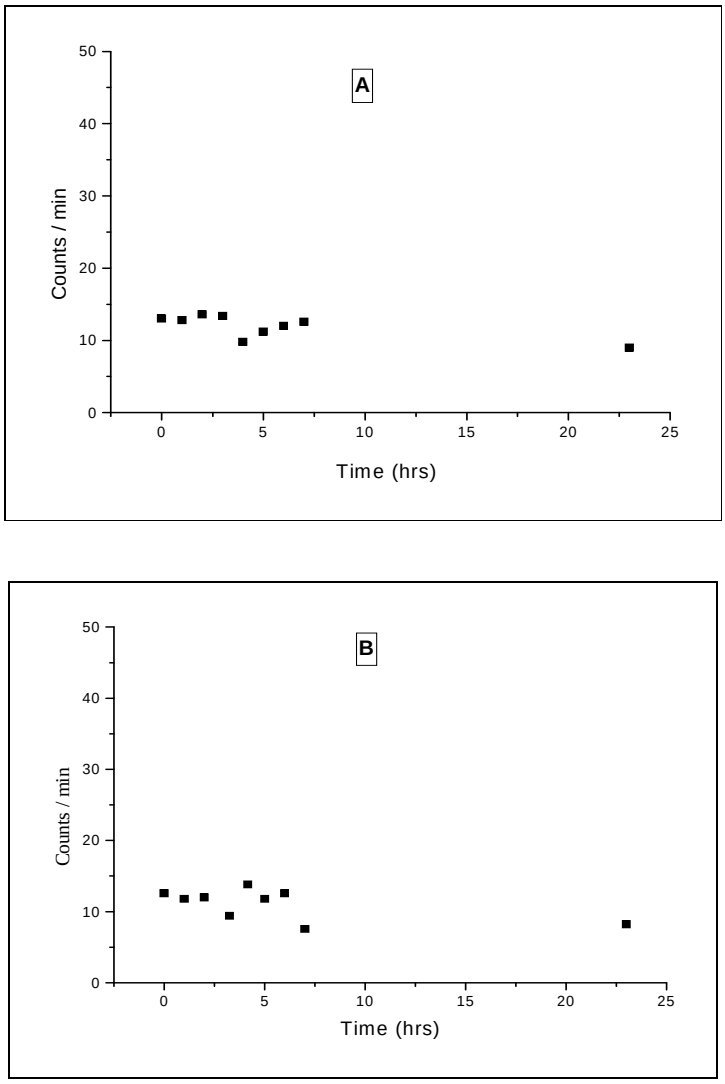


Fig. 5. Count rate of $^{14}$C-Chol transfer from donor liposomal membranes and serum components of FCS. Donor liposomes (EiPC/DCP/CHOL (7:1:2)) were loaded with $^{14}$C Chol and mixed with A) 10% FCS; B) 50% FCS .

Most mammalian cells require the addition of serum to the culture medium for optimal growth and maintenance of the cell lines *in vitro*. It is

important to understand the transfer process in the presence of serum to gain insight into the behavior of liposomes with the cells *in vivo*.

The effect of FCS on the cholesterol exchange rate between donor and acceptor vesicles is studied (Figs. 3 and 4). The half life time for cholesterol transfer in the presence of 10% FCS and 50% FCS were 7.63 hrs and 8.78 hrs, respectively. It was found that the presence of serum in the culture medium greatly diminished rather than increased the total transfer of liposomal lipid to the cells. These observations were taken to suggest that the diminished uptake of liposomal lipid may be caused by a modification of vesicles surface [11, 21].

There was no exchange or transfer from donor liposomes population to the serum components (Fig. 5). This means that the transfer has occurred from donor vesicles to acceptor vesicles only.

Despite the apparent simplicity of the experimental system and the current consensus as to its mechanism, we have found the analysis of the transfer of cholesterol between lipid compartments to be difficult and the kinetic patterns complex. Cholesterol transfer does not always proceed to completion (i.e. to isotopic equilibrium) in phospholipids vesicles [21].

Desorption of cholesterol from donor membranes appears to be rate-limiting for transport and is followed by aqueous diffusion of cholesterol to acceptor membranes [18]. It can be concluded that despite its extremely low solubility in water, cholesterol moves between lipid compartments by aqueous diffusion.

However, the models describing the spontaneous transfer from membrane to membrane of natural membrane components need to be considered to understand the drug migration between liposomes.

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