# THE SERUM LIPOPROTEINS AS A SOURCE OF MILK CHOLESTEROL

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## 1. Introduction

We have recently shown that [$^{14}$C] cholesterol placed in the abomasum (true stomach) of the lactating goat is transported to the milk [1]. Labeling of the very low density, low density and high density serum lipoproteins also was observed but the data did not permit any deductions as to which of them might be a precursor(s) of the milk cholesterol. In an effort to clarify this matter an experiment was conducted in which serum lipoproteins of the rat were labeled in vivo, isolated and individually evaluated in lactating rats for transfer of the labeled cholesterol to milk. It was found that all three of the lipoproteins transferred [$^{14}$C] cholesterol to milk and approximately in proportion to their total injected radioactivity.

## 2. Materials and methods

A non-pregnant, non-lactating female rat was fasted overnight and fed by stomach tube 50 $\mu$Ci of [4–$^{14}$C] cholesterol (New England Nuclear Corp., Boston, Massachusetts, U.S.A.), dissolved in 0.5 ml corn oil. Blood was collected by heart puncture after 6 h (fasting). This interval was found to yield maximum blood radioactivity by previous experiment. The following three classes of serum lipoproteins were prepared ultracentrifugally from this blood by methods employed in our earlier research with the goat [1]: VLDL, very low density lipoproteins, and chylomicrons $\rho<1.006$; LDL, low density lipoproteins, $1.006<\rho<1.063$; HDL, high density lipoproteins, $1.063<\rho<1.210$. Blood serum (5.65 ml) was diluted to 8 ml with 0.195 M NaCl containing 0.1 mg EDTA-$Na_2$ per ml for ultracentrifugal analysis.

Aliquots of the lipoprotein fractions were extracted to obtain the lipids according to the method of Folch et al. [2]. The extracts were dried under nitrogen, taken up in chloroform-methanol (2:1 v/v) and portions of the solution were separated into cholesterol and cholesteryl esters by thin layer chromatography on plates of silica gel G (Supelco, Bellefonte, Pennsylvania, U.S.A.). The solvent system was petroleum ether, ethyl ether and acetic acid 180:2:1 (v/v/v). The developed plates were placed briefly in an iodine saturated atmosphere to facilitate spot identification. Radioactivity was measured by scraping material from the appropriate plate areas into vials and counting in scintillation fluid. Cholesterol and cholesteryl esters were quantified by the method of Searcy and Bergquist [3].

The remainder of the ultracentrifugal density fractions were dialyzed against a mock serum solution (0.195 M NaCl) for 24 h at 4°C to remove accumulated salt. Each fraction was injected into the tail vein of a lactating rat. To facilitate milking the animals were anaesthetized with ethyl ether and injected intramuscularly with 0.1 USP units of oxytocin. Each animal was milked by aspirator suction every 6 h for 24 h. A further milking was obtained at 36 h. Milk samples were extracted and analyzed for cholesterol and cholesteryl ester radioactivity similarly to methods used for serum.

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Table 1
Radioactivity of cholesterol in serum lipoprotein preparations that were injected (i.v.) into lactating rats

| Serum lipoprotein | Cholesterol radioactivity (cpm/mg) | | cpm injected per rat |
|---|---|---|---|
| | Free | Esterified | |
| VLDL | 15 | 196 | 34 120 |
| LDL | 81 | 26 | 3472 |
| HDL | 85 | 23 | 25 104 |

## 3. Results and discussion

Table 1 summarizes the specific activity data for the serum lipoprotein fractions injected into the lactating rats. Fig.1 shows the transfer of cholesterol activity into free cholesterol of milk by the three lipoprotein preparations. Esterified cholesterol averaged 11.6% of the total milk cholesterol and contained less than 15% of the total radioactivity in the samples. During the first 24 h following injection the various lipoproteins transferred cholesterol radioactivity to milk approximately in proportion to the amounts of their total injected activities. According to Easter [4] the time to achieve maximum transfer of cholesterol radioactivity from serum to milk in the rat is 17 to 20 h. Thus it would appear that the data of fig.1 cover an appropriate period of time (36 h), and that preferential transfer of cholesterol from one serum lipoprotein to another cannot account for the relatively consistent proportionality of activity in milk cholesterol derived from the three lipoproteins.


Fig.1. Transfer of [$^{14}$C]cholesterol from serum to milk in lactating rats individually injected with cholesterol-labeled very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins. The scale at right shows relative specific activity of the lipoproteins at the time of injection with VLDL as 100.

In their study of dietary [$^{14}$C] cholesterol metabolism by the lactating rat Clarenburg and Chaikoff [5] found that milk cholesterol attained about 80% of the specific activity of plasma cholesterol. We observed a comparable relationship (70–75%) in the lactating goat during the 5 to 9 day period when the curves for serum and milk cholesterol activities were slowly declining [1]. This might suggest that the bulk of the milk cholesterol is derived from the circulation. However, Clarenburg and Chaikoff proposed that chylomicrons are a more or less specific vehicle for transport of serum cholesterol to milk and on that basis they estimated that roughly 80% of milk cholesterol is synthesized de novo in mammary tissue. In contrast our data (fig.1) suggest that rat serum may act as a fairly homogeneous source of milk cholesterol. The observed similarity in radioactivity relationships between serum and milk cholesterol for the rat and goat despite the comparative absence of chylomicrons from goat serum also suggests that the question of the serum source of milk cholesterol requires further appraisal. The possibility that all of the serum lipoproteins are capable of transferring cholesterol to tissues adjoining the circulation has significant implications to atherosclerosis.

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