Evidence for reverse cholesterol transport *in vivo* from liver endothelial cells to parenchymal cells and bile by high-density lipoprotein

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Acetylated low-density lipoprotein (acetyl-LDL), biologically labelled in the cholesterol moiety of cholesteryl oleate, was injected into control and oestrogen-treated rats. The serum clearance, the distribution among the various lipoproteins, the hepatic localization and the biliary secretion of the [³H]cholesterol moiety were determined at various times after injection. In order to monitor the intrahepatic metabolism of the cholesterol esters of acetyl-LDL *in vivo*, the liver was subdivided into parenchymal, endothelial and Kupffer cells by a low-temperature cell-isolation procedure. In both control and oestrogen-treated rats, acetyl-LDL is rapidly cleared from the circulation, mainly by the liver endothelial cells. Subsequently, the cholesterol esters are hydrolysed, and within 1 h after injection, about 60% of the cell-associated cholesterol is released. The [³H]cholesterol is mainly recovered in the high-density lipoprotein (HDL) range of the serum of control rats, while low levels of radioactivity are detected in serum of oestrogen-treated rats. In control rats cholesterol is transported from endothelial cells to parenchymal cells (reverse cholesterol transport), where it is converted into bile acids and secreted into bile. The data thus provide evidence that HDL can serve as acceptors for cholesterol from endothelial cells *in vivo*, whereby efficient delivery to the parenchymal cells and bile is assured. In oestrogen-treated rats the radioactivity from the endothelial cells is released with similar kinetics as in control rats. However, only a small percentage of radioactivity is found in the HDL fraction and an increased uptake of radioactivity in Kupffer cells is observed. The secretion of radioactivity into bile is greatly delayed in oestrogen-treated rats. It is concluded that, in the absence of extracellular lipoproteins, endothelial cells can still release cholesterol, although for efficient transport to liver parenchymal cells and bile, HDL is indispensable.

INTRODUCTION

The receptor-mediated uptake of acetylated low-density lipoprotein (acetyl-LDL) leads to massive deposition of cholesterol within phagocytotic cells [1,2]. The high-affinity binding sites for acetyl-LDL, i.e. scavenger receptors, have been implicated as a defence mechanism against the accumulation of modified lipoproteins in the blood [2]. In former publications we have demonstrated that acetyl-LDL (I-labelled in the apoprotein moiety) is almost completely cleared from the blood within 3 min of intravenous injection into rats [3]. This clearance is quantitatively exerted by the liver and especially by liver endothelial cells [4]. Studies with isolated macrophages in culture have indicated that the protein and cholesterol ester components are delivered intact to the lysosomes, where they are hydrolysed by cathepsins and acid lipase respectively [5]. The free cholesterol crosses the lysosomal membrane into the cytoplasm, where it can be re-esterified by the microsomal enzyme acyl-CoA:cholesterol acyltransferase (EC 2.3.1.26) and stored as cholesterol ester droplets in the cytoplasm [6]. Uptake of acetyl-LDL does not lead to a suppression of the scavenger receptor [2], and prevention of accumulation of cholesterol esters depends upon the presence of an effective acceptor, such as high-density lipoproteins (HDL), intact erythrocytes or lipoprotein-deficient serum fractions ($d > 1.21$) in the extracellular fluid [7]. Endothelial cells [8–10], as well as peritoneal macrophages [11,12] and fibroblasts [11], possess cell-surface sites that bind HDL reversibly with high

affinity. HDL binding can be induced by loading the cells with cholesterol derived from cholesterol/albumin complexes [10,11] or acetyl-LDL [8,12]. The cellular mechanism by which cholesterol is removed from the cells is in dispute, and two mechanisms have been suggested. First, receptor binding of HDL to the cholesterol-loaded cells may lead to internalization of the particle and interaction with cellular lipid droplets, after which cholesterol is resecreted from the cells into the medium [12]. Alternatively, the efflux of cholesterol from loaded cells may be a passive process in which cholesterol diffuses through the aqueous space to the lipoprotein [13,14]. Binding of HDL to its receptor does not influence this process, but appears to promote translocation of intracellular cholesterol to the plasma membrane [15,16].

The cholesterol-enriched HDL particle is expected to deliver the cholesterol to the liver hepatocytes and thus mediates reverse cholesterol transport to the liver [1]. Cholesterol can subsequently be excreted in bile and is thereby irreversibly removed from the circulation [17,18]. Although this sequence of events is generally accepted, evidence for this pathway *in vivo* is scarce [19]. Schwartz *et al.* have shown that the liver in man selectively utilizes and secretes free cholesterol from HDL for incorporation into biliary cholesterol [20].

Schmitz and co-workers [21,22] have suggested that, in the absence of HDL, another mechanism for the release of cholesterol may be effective. It appears that under these conditions secretion of cholesterol from macrophages is still possible, in the form of

Abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VHDL, very-high-density lipoprotein; VLDL, very-low-density lipoprotein; apo, apolipoprotein.

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lamellar bodies, which originate from lysosomes. In order to test *in vivo* whether the presence of HDL is obligatory for the release of cholesterol from liver endothelial cells and transport to parenchymal cells, we treated rats with pharmacological doses of 17 α -ethinyloestradiol for three days. Oestrogen treatment of rats induces LDL [apolipoprotein(apo)B,E] receptors on parenchymal cells [23,24], which also recognize apo-E-containing rat HDL [25]. This results in a large fall in plasma lipoprotein levels [23,26]. In the present study we injected acetyl-LDL, labelled in the cholesterol ester moiety with [3 H]cholesteryl oleate, into control and oestrogen-treated rats. After rapid initial uptake of acetyl-LDL, primarily by liver endothelial cells, we monitored the release of label from these cells and the appearance of this label in the blood, the various liver cells and the bile.

MATERIALS AND METHODS

Isolation, labelling and acetylation of LDL

Human LDL was isolated from blood of healthy volunteers as described by Redgrave *et al.* [27]. After density-gradient centrifugation, LDL ($1.024 < d < 1.055$) and lipoprotein-deficient serum ($d > 1.21$) were collected. [3 H]cholesteryl oleate was incorporated into LDL as described by Blomhoff *et al.* [28] exactly as specified earlier [29]. As reported before [29], the applied labelling procedure leads to an LDL preparation for which the serum decay rate is identical with that of iodinated LDL. The labelled LDL was acetylated according to the method of Basu *et al.* [30].

Bile sampling and determination of the serum clearance

Throughout this study, 3-month-old male Wistar rats, which had free access to food and water, were used. Rats were equipped with permanent catheters in the bile duct, the duodenum and the heart as described previously [31]. The bile duct and duodenum catheters were immediately connected to each other after the operation in order to maintain an intact enterohepatic circulation. The rats were allowed to recover from the operation for 1 week. Lipoproteins were introduced via the heart catheter. The bile duct was then connected to a fraction collector and bile was collected continuously into preweighed test tubes in 1 h fractions for 12 h, followed by 10 6-h fractions. Blood was sampled from the heart catheter at different times after injection. Colour was removed from bile samples by adding an equal volume of 30% H₂O₂, and radioactivity was counted after addition of scintillation fluid.

Cell isolation

For determination of the hepatic cellular distribution *in vivo*, the radiolabelled acetyl-LDL was injected into the tail vein of the rats. At the indicated time, the different cell types were isolated by a low-temperature cell-isolation technique, in such a way that liver parenchymal, endothelial and Kupffer cells could be isolated from the same rat. After the abdomen was opened, the vena porta was cannulated and the perfusion was started with Hanks' buffer, pH 7.4, at 8 °C. At 8 min after the perfusion was started, a lobule was tied off for determination of the total liver uptake. The perfusion was continued for 20 min with a Hanks' buffer containing 0.05% (w/v) collagenase and 1 mM-CaCl₂. Then the liver was cut into pieces in a plastic beaker in Hanks' buffer with 0.3% BSA, and filtered through a nylon gauze (mesh width 90 μ m). The filtrate was centrifuged for 30 s at 50 g four times, after which the final pellet consisted of 100% hepatocytes. The combined supernatants were centrifuged for 10 min at 400 g and the pellet (mainly non-parenchymal cells) was stored on ice. The material remaining on the nylon gauze filter was resuspended in Hanks' buffer containing 0.3% BSA and 0.25% Pronase and

incubated for 10 min at 8 °C. Then the cell suspension was centrifuged for 2 $\times$ 10 min at 400 g and the pellet (combined with the stored pellet) was suspended in Hanks' buffer. A further subdivision of the non-parenchymal cell preparation into an endothelial cell fraction and a Kupffer cell fraction was performed by centrifugal elutriation exactly as described earlier [4]. With this method, 2–3 mg of endothelial cell protein and 1–2 mg of Kupffer cell protein was obtained, which is a yield of 3–4.5% and 2–4% respectively, as we reported earlier [4]. The purity of each cell isolation was checked by light-microscopy after staining for peroxidase activity. Samples of the homogenized liver and the different liver cells were digested with Soluene-350 and counted for radioactivity.

Determination of cholesterol and cholesterol esters

In order to determine whether the [3 H]cholesterol is in the free or the esterified form, samples of the homogenized lobule, the different cell types and fractions of the density gradient were extracted according to Bligh & Dyer [32] and separated by t.l.c. as described earlier [33]. The mass of cholesterol and cholesterol esters in the fractions from the density gradient was determined by an enzymic colorimetric method using the CHOD-PAP kit from Boehringer.

Oestrogen treatment

Rats received a subcutaneous injection of 17 α -ethinyloestradiol in propylene glycol for 3 successive days at a dose of 5 mg/kg body weight.

Protein determination

Protein was determined according to Lowry *et al.* [34], with BSA as standard.

Statistics

Differences were tested for significance by Wilcoxon's two-sample test [35].

Chemicals

Collagenase type I and 17 α -ethinyloestradiol were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Pronase and the cholesterol (CHOD-PAP) kit were purchased from Boehringer, Mannheim, Germany; Nycodenz was from Nyegaard & Co A/S, Oslo, Norway, and [3 H(N)]cholesteryl oleate in toluene was from New England Nuclear, Dreieich, Germany. Soluene-350 was obtained from Packard.

RESULTS

Serum decay

The serum radioactivity was determined at different times after injection of [3 H]cholesteryl-oleate-labelled acetyl-LDL (3 H label as [3 H]cholesteryl-1,2,6,7- 3 H(N)]cholesteryl oleate) in control rats and in rats pretreated with oestrogens (Fig. 1). Oestrogen treatment caused a 80% decrease in serum cholesterol levels as compared with controls (0.47 ± 0.04 mM for oestrogen treatment versus 2.30 ± 0.10 mM for controls). The labelled acetyl-LDL was injected intracardially into the rats via a permanent heart catheter. The serum clearance of [3 H]cholesteryl-oleate-labelled acetyl-LDL is as rapid as that of acetyl-LDL labelled in its protein moiety [3]. In both control and oestrogen-treated rats, acetyl-LDL is cleared rapidly from the circulation, and 30 min after injection less than 1% of the injected radioactivity remained in the serum compartment. Thereafter, however, the serum radioactivity in the control rats increased again up to 5% of the injected dose and remained at this level for at least 24 h. In

Fig. 1. Serum decay of [^3H]cholesterol-ester-labelled acetyl-LDL in control and oestrogen-treated rats

[^3H]Cholesteryl-oleate-labelled acetyl-LDL ($300\text{ }\mu\text{g}$, 30 d.p.m./ng) was introduced intracardially via a permanent heart catheter in control ($\circ$, $n = 6$) and oestrogen-treated ($\bullet$, $n = 5$) rats. At different time points (first time points are 2 and 30 min) blood was obtained from the heart catheter and the serum was counted for radioactivity. Values are expressed as percentages of the injected dose (means $\pm$ S.E.M.). * Significantly different from control values ($P < 0.025$).

contrast, in the oestrogen-treated rats, the second phase is much less apparent, and the amount of radioactivity in serum remained below 1% of the injected dose.

Density-gradient analysis of the serum

Serum from control rats, obtained at different time intervals after injection of [^3H]cholesteryl-oleate-labelled acetyl-LDL, was subjected to density-gradient centrifugation. Fig. 2 shows that, at 3 min after injection, nearly all radioactivity present in the blood was recovered in the LDL region. In contrast, at 120 min after injection, the radioactivity was no longer associated with acetyl-LDL but was mainly recovered in the HDL range (for rats, this is $1.050 < d < 1.13$). At this time only 23.8% of the radioactivity was present in the LDL, 51.5% in the HDL fraction, 11.5% in the more-dense fractions ($d > 1.13$) and 13.2% in the very-low-density lipoprotein (VLDL) fraction ($d < 1.006$). Of the radioactivity in the HDL fraction at this time, 78% was in the form of free cholesterol. At longer time intervals after injection, the amount of radioactivity recovered in the HDL range increased, with the highest value at 9 h after injection (Fig. 2b). At this time point only 35% of the labelled cholesterol was in the free cholesterol form, indicating that a part of the labelled cholesterol is re-esterified, presumably by the action of the enzyme lecithin:cholesterol acyltransferase (EC 2.3.1.43). At 6 h after injection a part of the radioactivity appeared to be recovered in the very-high-density lipoprotein (VHDL) region (for rats, this is $1.13 < d < 1.21$). Between 24 and 48 h after acetyl-LDL injection the radioactivity in all fractions diminished again (Fig. 2c). In Fig. 3, the specific activities of the labelled cholesterol and cholesterol esters in the various density-gradient fractions of the serum and the erythrocytes are plotted against time. The specific activity for the esterified form of cholesterol could not be calculated for the erythrocytes and the VHDL fraction, because these fractions consisted almost totally of free cholesterol. It is clear that between 1 and 9 h after injection, the highest specific activity of [^3H]cholesterol is found in the HDL fraction. The time profile of cholesterol ester radioactivity in the HDL fraction is consistent with the conversion of cholesterol by lecithin:chol-

Fig. 2. Distribution of ^3H radioactivity in the KBr density gradient of serum obtained at different time intervals after injection of [^3H]cholesterol-oleate-labelled acetyl-LDL in control rats

[^3H]Cholesteryl-oleate-labelled acetyl-LDL ($150\text{ }\mu\text{g}$, 30 d.p.m./ng) was injected into the tail vein. At different times after injection, serum was obtained and a 3.6 ml sample was subjected to density-gradient centrifugation at 4°C . The gradient was subdivided into $500\text{ }\mu\text{l}$ fractions starting from the bottom. Time intervals after injection: (a) 3 ($\square$), 30 ($\blacktriangledown$), 60 (∇) and 120 ($\bullet$) min; (b) 2 ($\bullet$), 4 ($\blacktriangle$), 6 ($\times$) and 9 ($\blacksquare$) h; (c) 9 ($\blacksquare$), 24 ($\triangle$) and 48 ($\circ$) h. Values are expressed as ^3H radioactivity per fraction.

esterol acyltransferase, also leading to the highest specific activity of radioactive cholesterol esters in this fraction. In oestrogen-treated rats the distribution of the serum radioactivity among the various lipoproteins showed the same pattern as in the untreated animals (results not shown).

Hepatic uptake and processing of the labelled acetyl-LDL

In order to investigate the metabolic behaviour of the initially cell-associated radioactivity within the liver *in vivo*, the various liver cell types were isolated at different times after injection of

Fig. 3. Specific activities of [^3H]cholesterol (a) and [^3H]cholesterol ester (b) in the different fractions of the blood after injection of [^3H]cholesteryl-oleate-labelled acetyl-LDL in control rats

At different times after injection of [^3H]cholesteryl-oleate-labelled acetyl-LDL (150 μg , 30 d.p.m./ng) into rats, the serum was subjected to density-gradient centrifugation (see legend to Fig. 2). Values are expressed as counts of free or esterified cholesterol/ μg of free or esterified cholesterol (means $\pm$ S.E.M.) in the VLDL (Δ), HDL ($\bullet$), LDL ($\square$) and VLDL (Δ) fractions of the serum, and in erythrocytes ($\blacktriangledown$).

acetyl-LDL. The amount and form of the cell-associated radioactivity (Figs. 4 and 5) and the secretion of radioactivity into bile (Fig. 6) were measured. In control rats most of the radioactivity was initially found in the endothelial cells, which contained about 60% of the administered dose after 15 min. At this stage the parenchymal cells contained 13% of the label, whereas less than 5% of the injected dose was recovered in the Kupffer cells. At longer time intervals after injection of the acetyl-LDL, the distribution of radioactivity in the liver changed gradually. Between 15 and 30 min after injection the total radioactivity in the liver decreased, caused by a loss of radioactivity in the endothelial cells, while between 1 and 2 h after injection the total radioactivity in the liver increased again, due mainly to uptake of radioactivity by the parenchymal cells. Characterization of the label after lipid extraction indicates that the cholesterol esters were rapidly hydrolysed in the endothelial cells. By 30 min after injection nearly all of the cholesterol esters in this cell type were converted to free cholesterol. Subsequently the free cholesterol was gradually lost from the endothelial cells with an initial rapid phase representing 60% of the cell-associated label. The time-dependent behaviour of [^3H]cholesterol in parenchymal cells differs completely from its behaviour in endothelial cells. The small amount of [^3H]cholesteryl-oleate-labelled acetyl-LDL directly taken up by this cell type was also rapidly hydrolysed to free [^3H]cholesterol. However, this [^3H]cholesterol is not released rapidly from the cells, and a second increase in the amount of [^3H]cholesterol at 1–2 h after injection was observed. The small amount of acetyl-LDL taken up by Kupffer cells followed a similar pattern to the endothelial-cell-associated label.

In oestrogen-treated animals, almost the same pattern of distribution was seen initially as in control rats. The [^3H]cholesteryl oleate taken up by the endothelial cells was also rapidly hydrolysed and label was released from the cells. In this case the radioactivity was recovered in the serum compartment to a much lower extent than in control animals. Surprisingly, we noticed a rapid increase in [^3H]cholesterol radioactivity in Kupffer cells at 30–120 min after injection of labelled acetyl-LDL, which was not seen in the control animals. The radioactivity within the

Kupffer cells was again released between 2 and 9 h after injection. Erythrocytes showed no significant difference in the amount of [^3H]cholesterol under both conditions, and the amount of radioactivity recovered in the erythrocytes within 4 h after injection was $3.6 \pm 0.6\%$ of the injected dose for control rats and $4.4 \pm 0.7\%$ for oestrogen-treated rats.

Excretion of ^3H radioactivity into bile

Collection of bile was started immediately after administration of acetyl-LDL. The bile acid excretion rate rapidly decreases after interruption of the enterohepatic circulation to 5–7% of its initial value, due to exhaustion of the endogenous bile acid pool. After this period, bile acid excretion directly reflects hepatic synthesis rate [31]. In oestrogen-treated rats, bile flow was significantly lower due to a 40% decrease in the so-called bile-acid-independent fraction of the bile flow [18]. In contrast, bile acid synthesis is not significantly lower in oestrogen-treated rats [18].

The biliary excretion of ^3H radioactivity is illustrated in Fig. 6. The biliary excretion rate showed an initial peak in the first 1 h after injection of acetyl-LDL under both experimental conditions. This peak was probably caused by direct uptake and processing of [^3H]cholesteryl oleate acetyl-LDL by the parenchymal cells. In control rats a second peak was observed between 4 and 12 h after acetyl-LDL injection, reaching a maximal value (1.5% of dose/h) at 12 h after injection. In oestrogen-treated rats the first peak was similar to that from control rats; however, the second peak started at a later time point (9–12 h after injection) and the peak value was significantly lower (0.8% of dose/h) than in control rats.

Radioactivity in bile was mainly in the form of bile acids ($90.3 \pm 0.8\%$ and $88.7 \pm 1.3\%$ of the recovered amount at 12 h after injection in control and oestrogen-treated rats respectively). The remainder of biliary radioactivity consisted of free cholesterol. The ratio of [^3H]cholesterol to [^3H]bile acid was similar in control and oestrogen-treated animals (0.11 ± 0.02 and 0.13 ± 0.03 respectively).

The similar amount of radioactive bile acids appearing in the

Fig. 4. Determination of the processing of $[^3\text{H}]$ cholesteryl-oleate-labelled acetyl-LDL in the different liver cell types in control (○) and oestrogen-treated (●) rats

$[^3\text{H}]$ Cholesteryl-oleate-labelled acetyl-LDL ($150 \mu\text{g}$, 30 d.p.m./ng) was injected into the rats. At the indicated times after injection a low-temperature perfusion (8°C) was started. After 8 min a lobule was tied off for determination of the total liver uptake (a). Subsequently the parenchymal (b), endothelial (c) and Kupffer (d) cells were isolated at 8°C . Samples of the homogenized liver and the different cells were digested with Soluene-350 and counted for radioactivity (upper panels). Subdivision into cholesterol ester (CE) and free cholesterol (Chol) fractions were performed by lipid extraction and t.l.c. Significant increases in total and free cholesterol radioactivity were found in the liver and parenchymal cells between 1 and 2 h after injection ($P < 0.05$). Values are means $\pm$ S.E.M. * Significantly different from control values ($P < 0.05$).

Fig. 5. Relative contribution of different liver cell types to total uptake of [^3H]cholesteryl-oleate-labelled acetyl-LDL.

The percentage of the injected dose was calculated in the total liver and bile (●), parenchymal cells and bile (▲), endothelial cells (□) and Kupffer cells (▽), by multiplying the values from Fig. 4 by the amount of protein that each cell type contributes to total liver protein.

Fig. 6. Biliary excretion of ^3H radioactivity during 72 h after injection of [^3H]cholesteryl-oleate-labelled acetyl-LDL.

[^3H]cholesteryl-oleate-labelled acetyl-LDL (300 μg , 30 d.p.m./ng) was injected intracardially via a permanent heart catheter in control (○, $n = 6$) and oestrogen-treated (●, $n = 5$) rats. Bile was collected continuously for 72 h. Values are means $\pm$ S.E.M.; * significantly different from control values ($P < 0.025$).

bile during the first hours after injection in control and oestrogen-treated rats indicates that, despite the decrease in the bile flow in the oestrogen-treated rats, bile acid production in the liver parenchymal cells is not affected by this treatment. This indicates that the lower second peak in oestrogen-treated rats as compared with control rats cannot be explained by a direct effect of oestrogen on parenchymal bile acid formation.

DISCUSSION

In this study we describe the processing *in vivo* of cholesterol esters initially associated with acetyl-LDL. A biological method

was used for the incorporation of labelled cholesterol esters into acetyl-LDL [28,29]. The absence of cholesterol ester transfer protein activity in rat serum [36] enables studies on the hepatic uptake of the cholesterol ester as an integral component of acetyl-LDL. Hepatic cellular uptake sites for acetyl-LDL have been clearly established in previous studies with both iodine [3,4,28] and cholesteryl [^{14}C]oleate label [33].

In this paper we show that the cholesterol ester moiety of acetyl-LDL initially follows the same cellular uptake pattern as the apoprotein and becomes mainly associated with liver endothelial cells. Subsequently the cholesteryl oleate moiety is rapidly hydrolysed within 30 min and cholesterol, like oleate [33], is released from the cells. Of the initially cell-associated cholesterol about 60% was released within 1 h after injection. This is slightly more rapid than observed by Blomhoff *et al.* [28], who noticed just a small release in cholesterol radioactivity from the endothelial cells at 1 h after injection. However, as already stated by Blomhoff *et al.* [28], they could not draw conclusions about the precise kinetics of cholesterol ester hydrolysis and release, because in their studies both liver perfusion and cell separation were performed at 37 °C, leading to continuous processing of the cholesterol (esters). We performed a low-temperature cell isolation procedure in which metabolism is completely blocked during cell isolation. The cholesterol released from endothelial cells was specifically recovered in the HDL range of the serum and the radioactivity in this fraction increased up to 9 h after the initial injection as acetyl-LDL. Although the radioactivity in the HDL fraction at first was in the free cholesterol form, a part of the cholesterol was re-esterified within the HDL, starting at 2–4 h after injection. At 1–2 h after injection the [^3H]cholesterol radioactivity in the hepatocytes increased by 2-fold. As no [^3H]cholesterol-ester hydrolysis can occur inside the parenchymal cells in this time period, the [^3H]cholesterol is probably the consequence of transport from endothelial cells to the parenchymal cells. However, the kinetics do not exclude the possibility that transport in the blood occurs as [^3H]cholesterol ester followed by very rapid uptake and hydrolysis of incoming cholesterol esters in the parenchymal cells. Subsequently the [^3H]cholesterol is converted into ^3H -labelled bile acids and secreted into bile. This sequence of events of the processing of [^3H]cholesteryl-oleate-labelled acetyl-LDL, originally internalized by liver endothelial cells, provides evidence that transport of cholesterol from endothelial cells to liver parenchymal cells and bile *in vivo* can be mediated by HDL.

A second line of evidence that HDL is involved in transport of cholesterol from endothelial cells to liver parenchymal cells and bile *in vivo* was provided by studying the same sequence of events in oestrogen-treated rats. Apo-B,E receptors on liver parenchymal cells are induced by oestrogen treatment [23,24], and lipoprotein levels in the blood are lowered to about 20% of the control value. Treatment with 17 α -ethinyloestradiol does not alter the recognition sites for acetyl-LDL on the liver cells, nor does it accelerate uptake of this modified lipoprotein. Our data indicate that the initial hydrolysis of cholesterol esters in endothelial cells and release of [^3H]cholesterol from the endothelial cells occurs at the same rate as in control rats, despite the low amount of HDL in oestrogen-treated rats. Erythrocytes could not be responsible for this release as in both control and oestrogen-treated rats a similar percentage (4% of the injected radioactivity) was associated with erythrocytes. We suggest that the data indicate that HDL is not obligatory for release of [^3H]cholesterol from endothelial cells. It may be that, in the absence of HDL, cholesterol is released by a mechanism involving cholesterol-containing lamellar bodies [21,22]. Such lamellar bodies, which are composed mainly of phospholipids and free cholesterol, were shown by Schmitz and co-workers [21,22] to be

secreted into the surrounding medium of macrophages in the absence of cholesterol acceptors. It is possible that similar lamellar bodies are also formed in the oestrogen-treated rats and, once secreted into the blood, are rapidly cleared from the blood by the Kupffer cells. Such a mechanism would explain the secondary increase of [^3H]cholesterol in Kupffer cells as observed in oestrogen-treated rats. Whatever the alternative mechanism may be, it is clear that the secondary appearance of radioactivity in bile is greatly inhibited under these conditions, so providing evidence *in vivo* that HDL is obligatory for an efficient transport of [^3H]cholesterol from endothelial cells to liver parenchymal cells and bile.

Oestrogen treatment is known to increase the activity of acyl-CoA:cholesterol acyltransferase [29,37] and to decrease that of 7α -hydrolase [37,38]. However, the absence of any effect of oestrogen treatment on the appearance of radioactivity in bile up until 3 h after injection of [^3H]cholesteryl-oleate-labelled acetyl-LDL indicates that the direct formation of radioactive bile acids in parenchymal cells is not influenced by oestrogen treatment.

The question of whether a certain subfraction in HDL is responsible for [^3H]cholesterol release from endothelial cells and transport to parenchymal cells cannot be answered by our study. Although we observed an initial rise in the density range $1.050 < d < 1.150$, which precedes the rise in the VHDL fraction ($1.150 < d < 1.230$), we cannot exclude the possibility that a small subfraction in HDL [39] or VHDL serves as an acceptor with rapid transfer to the large HDL pool.

Liver uptake of modified lipoproteins from the serum protects the body from an excessive accumulation of cholesterol esters in macrophages at other sites in the body and their possible conversion into foam cells. Such a protection mechanism will only be efficient if uptake is coupled to a complete processing of the modified lipoproteins and secretion of the metabolic products into bile. The present data indicate that, *in vivo*, adequate levels of HDL are necessary to exert such a function efficiently.

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