Selective uptake of cholesteryl esters from apolipoprotein-E-free high-density lipoproteins by rat parenchymal cells *in vivo* is efficiently coupled to bile acid synthesis

Moniek N. PIETERS,*† Donald SCHOUTEN,* Hille F. BAKKEREN,* Bas ESBACH,† Adriaan BROUWER,† Dick L. KNOOK† and Theo J. C. VAN BERKEL*

*Division of Biopharmaceutics, Center for Bio-Pharmaceutical Sciences, Sylvius Laboratory, University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands, and †Institute for Aging and Vascular Research, IVVO-TNO Leiden, Zernikedreef 9, 2333 CK Leiden, The Netherlands

[³H]Cholesteryl ester-labelled human high-density lipoprotein (HDL) was injected into rats and its decay, intrahepatic cellular distribution and the kinetics of biliary secretion were determined. At 10 min after injection the hepatic uptake of cholesteryl esters from HDL was 3-fold higher as compared with the apolipoprotein. Selective uptake was exerted only by parenchymal cells (5.6-fold more cholesteryl esters than apolipoprotein) and not by liver endothelial or Kupffer cells. The kinetics of biliary secretion of processed cholesteryl esters initially associated with HDL or low-density lipoprotein (LDL) were compared in unrestrained rats, equipped with permanent catheters in bile duct, duodenum and heart. At 72 h after injection of [³H]cholesteryl oleate-labelled HDL, $51.0 \pm 2.5\%$ of the injected dose was recovered as bile acids, which is about twice as high as the secretion of biliary radioactivity after injection of [³H]cholesteryl oleate-labelled LDL. Oestradiol treatment stimulated only liver uptake of LDL cholesteryl esters, and resulted in a 2-fold higher liver uptake than with HDL. However, the rate of radioactive bile acid formation from [³H]cholesteryl oleate-labelled HDL was still more rapid than for LDL. It is concluded that the selective uptake pathway for cholesteryl esters from HDL in parenchymal cells is more efficiently coupled to the formation of bile acids than is the cholesteryl ester uptake from LDL. This efficient coupling may facilitate the role of HDL in reverse cholesterol transport.

INTRODUCTION

The inverse correlation between plasma high-density lipoprotein (HDL) cholesterol levels and the incidence and prevalence of atherosclerosis has led to the suggestion that HDL plays a protective role against atherogenesis. According to the generally accepted concept of Glomset [1], HDL takes up cholesterol from peripheral cells and delivers it to the liver. The biliary secretion of cholesterol, either in unchanged form or as bile acids, is the major route in the body for eliminating cholesterol from the circulation. Cholesterol and cholesteryl esters are delivered to the liver by various lipoproteins, and the influence of lipoproteins on bile acid biosynthesis and secretion have been studied *in vitro* as well as *in vivo*.

In isolated rat hepatocytes, very-low-density lipoproteins (VLDL) and chylomicron remnants [2,3], but also apolipoprotein (apo) E-rich rat HDL [4,5], have been reported to increase bile acid synthesis. Addition of apo E-free HDL had no effect on bile acid synthesis. The uptake of apo E-rich HDL cholesterol was probably mediated by the remnant or low-density lipoprotein (LDL) receptor, since methylation of HDL abolished its capacity for stimulating bile acid synthesis [5]. In a recent study with rat hepatocytes, Junker & Davis [6] demonstrated that both apo E-free LDL and apo E-rich HDL were able to stimulate bile acid synthesis to the same extent. Binding of apo E-free LDL could be competed for by apo E-rich HDL, and vice versa, thus suggesting a LDL-receptor-mediated pathway. Both LDL and apo E-containing HDL also stimulated bile acid synthesis in cultured rabbit hepatocytes [7]. Cholesteryl esters rather than unesterified cholesterol of apo E-containing HDL were metabolized into bile acids by chick-embryo hepatocytes [8].

Studies *in vivo* in humans, in which radiolabelled HDL and LDL were administered to subjects with a bile fistula, showed that free cholesterol from HDL is the major substrate for biliary cholesterol [9]. Recently it has been reported that, in rats, free cholesterol from HDL₂ rather than from HDL₃ is preferentially utilized for bile acid synthesis [10]. However, interpretation of these studies *in vivo* is hampered by the fact that unesterified cholesterol readily exchanges between lipoproteins or membrane surfaces. In the present study we have labelled HDL in the cholesteryl ester moiety and studied the kinetics of liver uptake and biliary secretion in the rat. The rat does not possess an active cholesteryl ester transfer protein [11], and therefore the label will remain associated with the HDL particle in the circulation. Furthermore, we have administered relatively low amounts of labelled lipoproteins so that no mass changes in bile acid synthesis were induced. The data are compared with the characteristics of LDL uptake and bile acid formation.

MATERIALS AND METHODS

Isolation and labelling of HDL

Human HDL was isolated from the blood of healthy volunteers by differential ultracentrifugation as described by Redgrave *et al.* [12]. HDL ($1.063 < d < 1.21$) was dialysed against 8 mm-phosphate-buffered saline/1 mM-EDTA, pH 7.4, and labelled with [³H]cholesteryl oleoyl ether or [³H]cholesteryl oleate (Amersham Corp., Arlington Heights, IL, U.S.A.) by exchange from donor particles. The method, as specified below, is a modification of the procedure originally described by Pittman *et al.* [13].

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

† To whom correspondence should be addressed.

Donor ^3H -labelled particles

Egg-yolk phosphatidylcholine (Fluka, Buchs, Switzerland) and cholesteryl oleate (BDH Chemicals, Poole, Dorset, U.K.) were added in chloroform/methanol (1:1, v/v) in a mass ratio of 60:1 to a 25 ml glass scintillation vial and dried under a stream of N_2 . Then 250 μCi of cholesteryl [$1\alpha,2\alpha(n)$ - ^3H]oleate or cholesteryl [$1\alpha,2\alpha(n)$ - ^3H]oleoyl ether (Amersham) was added to the mixture, dried under N_2 and placed overnight in a vacuum desiccator to remove residual solvent. Then 7.5 ml of 0.1 M-KCl/10 mM-Tris/1 mM-EDTA/0.025 % $\text{Na}_2\text{S}_2\text{O}_3$ buffer, pH 8.0, was added, flushed with N_2 and sonicated with a MSE Soniprep 150 for 40 min (amplitude 12 μm) at 52 °C under a constant stream of N_2 . The microemulsion was adjusted to a density of 1.21 g/ml and subsequently subjected to density-gradient ultracentrifugation (Redgrave *et al.* [12]) in order to isolate donor particles with a density of approximately 1.03 g/ml. Phospholipid content of the particles was measured by an enzymic colorimetric assay (Phospholipids Kit, from Boehringer, Mannheim, Germany).

Labelling of HDL

A 2 ml portion of human lipoprotein-deficient serum, extensively dialysed against 8 mM-phosphate-buffered saline/1 mM-EDTA, was added to human HDL (1 ml, containing approx. 3.5 mg of protein) and donor particles. The mass ratio of HDL protein to donor-particle phospholipids was 8:1. The mixture was then incubated for 5 h at 37 °C in a shaking water bath under N_2 . To separate HDL from the donor particles, the mixture was raised in density to 1.21 g/ml with KBr and subjected to differential ultracentrifugation. After re-isolation, the radiolabelled HDL was dialysed and passed through a heparin-Sepharose affinity column to remove apo E [14]. The apo E-free fraction was checked for the presence of apo E and albumin by SDS/PAGE with 5–22.5 % -acrylamide gels, followed by Coomassie Blue staining. Almost all of the radioactivity could be recovered in the cholesteryl ester moiety of HDL as determined by Bligh & Dyer extraction [21] and t.l.c.: 1.0 ± 0.1 % and 2.0 ± 0.1 % were recovered as ^3H -labelled unesterified cholesterol for labelling with [^3H]cholesteryl oleoyl ether and [^3H]cholesteryl oleate respectively. [^3H]cholesteryl ester- or ether-labelled HDL was checked for phospholipid, cholesterol and cholesteryl ester content (phospholipids kit and CHOD-PAP kit; Boehringer Mannheim), density and electrophoretic α -mobility. Iodinated [^3H]cholesteryl ester or ether HDL had the same plasma decay kinetics and liver association as reported previously [15]. HDL was iodinated by the IDI method of McFarlane [16], as modified by Bilheimer *et al.* [17]. In negatively stained preparations the labelled HDL had the same appearance as its unlabelled counterpart.

Oestradiol treatment

When indicated, rats received for 3 successive days a subcutaneous injection of 17α -ethinyl oestradiol (Sigma, St. Louis, MO, U.S.A.) in propylene glycol at a dose of 5 mg/kg body wt.

Serum decay *in vivo* and liver association

Rats were anaesthetized by intraperitoneal injection of Nembutal (1 ml/kg body wt.). After the abdomen was opened, radiolabelled HDL was injected into the vena penis. Blood sampling and liver lobule excision were performed as described previously [18].

Tissue distribution

Rats were anaesthetized with diethyl ether, and radiolabelled HDL was administered via the vena penis. After 4 or 24 h rats

were killed, individual tissues weighed and samples combusted in a Packard Sample Oxidizer 306. The samples were counted for radioactivity and corrected for the contribution of the serum to the total counts as described previously [18].

Cell isolation

The hepatic cellular distribution was studied by using a low-temperature cell-isolation technique as described previously [18]. After injection of radiolabelled HDL in the vena penis, the vena porta was cannulated at the indicated time and the liver was perfused with oxygenated Hanks' buffer, pH 7.4, at 8 °C. To determine total liver uptake, a lobule was tied off 8 min after starting the perfusion. The perfusion was continued for 20 min with Hanks' buffer containing 0.05 % (w/v) collagenase and 1 mM- CaCl_2 . Parenchymal cells were isolated by mincing the liver in Hanks' buffer with 0.3 % BSA, filtering it through a nylon gauze and centrifuging 4×30 s at 50 g. The pellet consisted of pure parenchymal cells, as judged by examination by light-microscopy. The non-parenchymal cells present in the supernatants were centrifuged for 10 min at 400 g and stored on ice. The remainder on the nylon gauze was incubated with Hanks' buffer containing 0.3 % BSA and 0.25 % Pronase for 10 min at 8 °C. The cell suspension was centrifuged at 400 g for 10 min and the pellet combined with the stored non-parenchymal cell pellet. By centrifugal elutriation the non-parenchymal cells were subdivided into an endothelial-cell fraction and a Kupffer-cell fraction [19]. The purity of each cell fraction was checked by light microscopy after staining for peroxidase activity, and samples were counted for radioactivity.

Bile sampling

Bile was collected from unrestrained 3-month-old male Wistar rats, as reported previously [20]. Rats received tap water and standard chow *ad libitum*. Rats were equipped with permanent catheters in the bile duct, the duodenum and the heart. In order to maintain an intact enterohepatic circulation, the bile duct and duodenum catheters were connected immediately after surgery. Rats were allowed to recover for 1 week. Lipoproteins were introduced via the heart catheter. The bile-duct catheter was then connected to a fraction collector, and bile was collected for 72 h. Bile samples were counted for radioactivity after combustion in a Packard sample oxidizer 306.

In order to separate bile acids from cholesterol and cholesteryl esters, samples were extracted as described by Bligh & Dyer [21]. The aqueous layer containing bile acids was counted for radioactivity. Cholesterol and cholesteryl esters were separated by t.l.c. with as developing solvent heptane/diethyl ether/acetic acid (60:40:1, by vol.). Spots were made visible by colouring with I_2 , scraped off and counted for radioactivity.

Protein determination

Protein was determined as described by Lowry *et al.* [22], with BSA as standard.

RESULTS

Serum decay

Fig. 1 depicts the decay in serum of iodinated HDL and [^3H]cholesteryl ester- or [^3H]cholesteryl oleoyl ether-labelled HDL. The cholesteryl ester- or cholesteryl ether-labelled moieties of HDL both follow a similar monoexponential decay, and are cleared much faster than the iodinated apolipoproteins. These findings are in accordance with earlier published data [23–25] and are indicative of a similar selective removal of HDL cholesteryl esters or ethers from the circulation.

Fig. 1. Serum decay of apo E-free [^3H]cholesteryl oleoyl ether-labelled, [^3H]cholesteryl oleate-labelled HDL and apo E-free ^{125}I -apoprotein-labelled HDL

Apo E-free [^3H]cholesteryl oleoyl ether-labelled HDL ($\bullet$) (250 000 d.p.m., 125 μg), [^3H]cholesteryl oleate-labelled HDL ($\circ$) (250 000 d.p.m., 125 μg) or ^{125}I -HDL ($\square$) (25×10^6 c.p.m., 100 μg) was injected into the vena penis of anaesthetized rats. At the indicated time points serum values were obtained, and the data were expressed as percentages of injected dose.

Tissue distribution

Tissue-distribution studies at 24 h after injection of [^3H]cholesteryl ether-labelled HDL (Fig. 2a) indicate that the liver is the major site of cholesteryl ether accumulation. At the same time point the liver-associated [^3H]cholesteryl ester-labelled HDL is significantly lower and is accompanied by an increase in radioactivity in the small and large intestines (Fig. 2b). The relative importance of the liver as the initial site of uptake is, however, evident at 4 h after injection (Fig. 2c). These data are consistent with a primary hydrolysis of cholesteryl esters in the liver, which is necessary for the subsequent secretion in the intestine, presumably as radiolabelled bile. Total liver association, calculated as the sum of liver, small-intestine- and large-intestine-associated radioactivity, was identical for [^3H]cholesteryl ester- ($57.8 \pm 7.6\%$) and [^3H]cholesteryl ether- ($62.5 \pm 3.5\%$) labelled HDL.

Intrahepatic cellular distribution of [^3H]cholesteryl ester HDL

To identify the intrahepatic uptake sites for [^3H]cholesteryl ester-labelled HDL, the various liver cell types, parenchymal, Kupffer and liver endothelial cells, were isolated at 10 min after injection. With total liver, the association of the cholesteryl ester moiety of HDL was 3-fold higher than for the apolipoprotein moiety (Fig. 3). The uptake of HDL cholesteryl esters in parenchymal cells was 5.6 times the apolipoprotein uptake, but no significant difference between HDL cholesteryl esters and apolipoprotein was observed with liver endothelial and Kupffer cells.

Fig. 2. Recovery sites of ^3H radioactivity at 24 h after injection of [^3H]cholesteryl oleoyl ether-labelled HDL (a) and at 4 h (c) and 24 h (b) after injection of [^3H]cholesteryl oleate-labelled HDL

Radiolabelled HDL (500 000 d.p.m., 250 μg) was injected into control rats. At the indicated time, rats were killed and tissues weighed and sampled. Tissue samples were corrected for serum contribution. Values are means $\pm$ S.E.M. of three experiments and are expressed as percentage of injected dose. Abbreviation: int, intestine.

The relative contribution of each liver cell type to the total uptake by the liver can be calculated by using the specific uptake/mg of cell protein and taking into account their relative contributions to total liver protein [26] (Table 1). The great majority of the cholesteryl esters from HDL appears to be directed to parenchymal cells. The total contribution by liver endothelial cells and Kupffer cells is relatively low and significantly less than for the apoprotein moiety, as a consequence of the absence of preferential cholesteryl ester uptake in these cell types. At 10 min after injection no difference in cellular uptake was observed between [^3H]cholesteryl oleate-labelled or [^3H]cholesteryl oleoyl ether-labelled HDL (results not shown).

Fig. 3. Distribution *in vivo* of apo E-free [³H]cholesteryl ester-labelled HDL and ¹²⁵I-HDL between parenchymal, liver endothelial and Kupffer cells, 10 min after injection

At 10 min after administration of [³H]cholesteryl ester-labelled HDL, the liver was perfused and parenchymal cells (PC), endothelial cells (EC) and Kupffer cells (KC) were isolated with collagenase and Pronase at 8 °C. Values are expressed as % of injected dose ($\times 10^{-3}$)/mg of cell protein for [³H]HDL ($\square$) (2×10^6 d.p.m., 1000 μ g) or ¹²⁵I-HDL ($\boxplus$) (10×10^6 c.p.m., 40 μ g) [15].

Table 1. Relative contributions of parenchymal, liver endothelial and Kupffer cells to the total uptake of apo E-free [³H]HDL and apo E-free ¹²⁵I-HDL by the rat liver

Relative contributions of parenchymal cells, liver endothelial cells and Kupffer cells to the total uptake of [³H]HDL (2×10^6 d.p.m., 1000 μ g) and ¹²⁵I-HDL (10×10^6 c.p.m., 40 μ g) [15] was calculated by taking into account the contribution of each liver cell type to the total liver protein, which are 92.5%, 3.3% and 2.5% for parenchymal, endothelial and Kupffer cells respectively. Values are means $\pm$ S.E.M. of three experiments.

Cell type	Percentage of total liver-associated radioactivity	
	[³ H]HDL	¹²⁵ I-HDL
Parenchymal	90.8 $\pm$ 0.7	77.8 $\pm$ 2.4
Endothelial	4.4 $\pm$ 0.3	10.8 $\pm$ 0.8
Kupffer	5.2 $\pm$ 0.8	11.3 $\pm$ 1.7

Biliary secretion of ³H radioactivity in control rats

In order to investigate the kinetics of the secretion of initial HDL-associated cholesteryl esters into bile, rats were equipped with permanent catheters in bile duct, duodenum and heart. The liver uptake and kinetics of appearance of biliary radioactivity were compared with data obtained for human LDL. In rats, human LDL has the same metabolic behaviour as homologous LDL with respect to catabolic sites and rate of liver uptake [27] which validates the use of human LDL in the rat. The liver association of [³H]cholesteryl oleate-labelled HDL as compared with [³H]cholesteryl oleate-labelled LDL in control rats is higher (at 4 h after injection, 3-fold higher) (Fig. 4). At 24 h after injection the liver association of [³H]HDL radioactivity is decreased.

Fig. 4. Liver association of [³H]cholesteryl ester-labelled HDL and [³H]cholesteryl ester-labelled LDL in control rats

Liver association of [³H]cholesteryl oleate-labelled HDL ($\circ$) ($250\,000$ d.p.m., 125 μ g) and [³H]cholesteryl oleate-labelled LDL ($\square$) ($800\,000$ d.p.m., 50 μ g) [28] was determined in control rats at the indicated time points. Values are corrected for serum contribution and are means $\pm$ S.E.M. of three experiments.

Fig. 5. Biliary secretion of ³H radioactivity after administration of [³H]cholesteryl oleate-labelled HDL ($\circ$) or [³H]cholesteryl oleate-labelled LDL ($\square$) in control rats

Values are expressed as percentage of injected dose per h (a) and as cumulative percentage of injected dose (b). Bile was collected for 72 h at time intervals of 1 h after injection of [³H]cholesteryl oleate-labelled HDL ($500\,000$ d.p.m., 250 μ g) into unrestrained control rats ($\circ$) and compared with values for [³H]cholesteryl oleate-labelled LDL (8×10^6 d.p.m., 500 μ g) ($\square$) [28,29]. Values represent means $\pm$ S.E.M. of four experiments. Black horizontal bars indicate the dark periods. For clarity in Fig. 5(a), data points between 12 and 72 h after injection are only presented every 4 h.

The secretion of biliary radioactivity after the administration of radiolabelled lipoprotein in the heart catheter of the rat, expressed as percentage of injected dose/h, is relatively constant

Fig. 6. Comparison of the biliary secretion of ^3H radioactivity after administration of [^3H]cholesteryl oleate-labelled HDL (○) or [^3H]cholesteryl oleoyl ether-labelled HDL (●)

Radiolabelled HDL (500 000 d.p.m., 250 μg) was injected intracardially into unrestrained catheterized rats. Bile was collected immediately after injection. Secretion is cumulatively expressed as a percentage of the injected dose. Values represent means $\pm$ S.E.M. of four experiments.

Fig. 7. Liver association of [^3H]cholesteryl ester-labelled HDL and [^3H]cholesteryl ester-labelled LDL in oestradiol-treated rats

Liver association of [^3H]cholesteryl oleate-labelled HDL (●) (250 000 d.p.m., 125 μg) or [^3H]cholesteryl oleate-labelled LDL (■) (800 000 d.p.m., 125 μg) [28] was determined in oestradiol-treated rats. Values are corrected for serum contribution and are means $\pm$ S.E.M. of three experiments.

during the first 6 h (Fig. 5a). A marked increase in secretion rate is noticed during the dark period. During the first 24 h after lipoprotein injection, the rate of appearance of radioactivity originating from HDL is about twice that with LDL. The day/night variation is also evident with LDL. The higher rate of radioactivity secretion leads to a higher cumulative recovery of radioactivity in bile from [^3H]cholesteryl ester-labelled HDL as compared with LDL (Fig. 5b). At 72 h after injection of [^3H]HDL, 51% of the injected dose was secreted in the bile, whereas with [^3H]LDL 30% of the radioactivity was recovered in the bile [28,29]. Bile analysis revealed that both for LDL and HDL the radioactivity in the bile was mainly present in the form of bile acids ($94.2 \pm 3.3\%$).

In agreement with the low amount of radioactivity recovered in the intestine after administration of [^3H]cholesteryl ether-labelled HDL (Fig. 2a), radioactivity from [^3H]cholesteryl oleoyl ether-labelled HDL appeared in the bile at a slow rate. At 24 h only 4.3% of the injected dose was recovered in the bile, whereas with [^3H]cholesteryl oleate-labelled HDL biliary secretion

Fig. 8. Biliary secretion of ^3H radioactivity after administration of [^3H]cholesteryl oleate-labelled HDL (●) or [^3H]cholesteryl oleate-labelled LDL (■) into oestradiol-treated rats

Values are expressed as percentages of injected dose per h (a) and as cumulative percentage of injected dose (b). Bile was collected for 72 h in time intervals of 1 h after injection of [^3H]cholesteryl oleate HDL (500 000 d.p.m., 250 μg) in unrestrained oestradiol-treated rats (●) and compared with values of [^3H]cholesteryl oleate-labelled LDL (■) (8×10^6 d.p.m., 500 μg) [28,29]. Values represent means $\pm$ S.E.M. of three experiments. Black horizontal bars indicate the dark periods. For clarity in Fig. 8(a), data points between 12 and 72 h are only presented every 4 h.

reached 26.5% of the injected dose (Fig. 6). With the rats injected with cholesteryl oleoyl ether-labelled HDL, the radioactivity in bile was mainly present as unchanged cholesteryl ethers, and no hydrolysed products or bile acids were found.

Biliary secretion of ^3H radioactivity in oestradiol-treated rats

In earlier studies [28,29] we have shown that initial uptake of LDL in Kupffer cells in the rat is not efficiently coupled to bile acid synthesis and secretion. The major part of [^3H]cholesteryl ester-labelled HDL is, however, directed to parenchymal cells (Table 1). In order to allow a comparison of the rate of processing of cholesteryl esters derived from LDL and HDL in parenchymal cells, we have treated rats with ethinyl oestradiol. Oestradiol treatment selectively induces apo B,E receptors on parenchymal cells [30]. With [^3H]cholesteryl ester-labelled LDL we have shown previously that oestradiol treatment enhances specifically LDL cholesteryl ester uptake in parenchymal cells, and that the processing of radiolabelled cholesteryl esters to bile acids is then coupled very efficiently to liver uptake [28]. In Fig. 7 the liver association of [^3H]HDL and [^3H]LDL in oestradiol-treated rats is compared. Oestradiol treatment strongly stimulates the liver association of LDL, whereas liver association of radioactivity from [^3H]HDL is hardly affected. The elevated liver association of LDL is accompanied by a higher rate of biliary ^3H secretion (Fig. 8a). When [^3H]HDL is injected into oestradiol-treated rats,

an initial high secretion rate is observed. The latter cannot be explained by an increased association of HDL with the liver, since serum decay and liver association of [^3H]HDL and [^{125}I]HDL (results not shown) hardly changed in oestradiol-treated rats. The cumulative appearance of radioactivity in the bile is greatly enhanced for LDL in oestrogen-treated rats (55 % at 72 h after injection, versus 30 % in control rats; Fig. 8b). However, it appears that, although the liver association of [^3H]HDL is about half of that of [^3H]LDL in oestradiol-treated rats, biliary secretion of radioactivity from [^3H]HDL still occurs at a higher initial rate than with LDL (Fig. 8a).

DISCUSSION

Selective uptake of HDL cholesteryl esters without the parallel uptake of its apoprotein moiety has been reported in a variety of cell types *in vivo* and *in vitro* [13,24,31]. Selective uptake of HDL cholesteryl esters has been shown to occur in hepatic [13,23–25,32,33], extrahepatic [34] and steroid-producing tissues [23,24,31,35] and could be down-regulated by cholesterol loading of the cell [34,36]. The liver is the major site of cholesteryl ester uptake, and in the present study we show *in vivo* that the selective uptake in the liver is exerted only by the parenchymal cells. At 10 min after injection, the uptake of HDL cholesteryl esters in parenchymal cells is 5.6 times the apoprotein uptake ($P < 0.0001$, Student's *t* test). In contrast, liver endothelial and Kupffer cells showed no significant difference between cholesteryl ester and apolipoprotein uptake. It can be calculated that parenchymal cells were responsible for 90.8 % of HDL cholesteryl ester uptake in the liver.

High-affinity binding sites have been identified on liver cells of various species [37–42]. Recently we have shown that specific high-affinity binding sites for HDL are present on rat parenchymal, liver endothelial and Kupffer cells [15] as well as on human parenchymal cells [43]. The binding properties of parenchymal, liver endothelial and Kupffer cells for apo E-free HDL appeared to be identical for the three cell types [15]. In the present study we show that the mechanism by which HDL delivers cholesteryl esters to parenchymal, liver endothelial and Kupffer cells is clearly different. Selective uptake of HDL cholesteryl esters occurs in parenchymal cells, whereas liver endothelial cells and Kupffer cells appear to take up HDL cholesteryl esters in parallel with the apoprotein. It can be concluded that, although the initial recognition site for HDL on various cells may be identical, the selective uptake of HDL cholesteryl esters must depend on HDL-binding independent factors. It can be expected that the high-affinity binding of HDL to cells will be a prerequisite for selective uptake, but that additional specific cellular properties are needed for selective cholesteryl ester uptake.

According to the generally accepted concept of Glomset [1], HDL is expected to deliver cholesterol (esters) from peripheral cells to the liver, in which cholesterol can be converted into bile acids and irreversibly removed from the circulation. Although this sequence of events is generally accepted, evidence for this pathway *in vivo* is scarce [44]. In the literature, data are available concerning the processing of HDL labelled in its unesterified cholesterol [9,10]. Free cholesterol, however, rapidly exchanges between plasma membranes and lipoproteins. In our experiments we have used human apo E-free HDL labelled in its cholesteryl ester moiety. Because rat plasma lacks functional cholesteryl ester transfer activity [11], the label remains associated with the particle until it is cleared from the circulation. To quantify the conversion of HDL cholesteryl esters into biliary products, we have used unrestrained catheterized rats. It appears that *in vivo* [^3H]cholesteryl ester-labelled HDL is actively processed by the

liver. At 72 h after administration, 51 % of the injected dose was recovered in the bile in the form of bile acids. This is about twice the amount of secreted radioactivity as was obtained with [^3H]cholesteryl ester LDL [28]. Furthermore, hydrolysis of cholesteryl esters appeared to be necessary for biliary secretion, since with [^3H]cholesteryl oleoyl ether-labelled HDL the secretion of radioactivity in the bile appeared to be very slow, and at 24 h after administration only 4.3 % of the injected dose was recovered in the bile.

In earlier studies [28,29] we have shown that initial uptake of LDL in Kupffer cells in the rat is not efficiently coupled to bile acid synthesis and secretion. To compare the kinetics of processed cholesteryl esters initially associated with LDL and HDL, we have also used oestradiol-treated rats. In oestradiol-treated rats more than 90 % of both HDL and LDL cholesteryl esters are directed to parenchymal cells. The liver association of HDL cholesteryl esters is hardly affected by oestradiol treatment. It appears that the liver association of cholesteryl esters from LDL in oestradiol-treated rats is 2-fold higher than for HDL. However, the secretion of labelled bile acids after injection of [^3H]HDL is initially twice as high as for LDL. This indicates that the selective uptake pathway for cholesteryl esters from HDL is apparently more efficiently coupled to the formation of bile acids than is that with LDL. Studies by Havel *et al.* have indicated that the LDL-receptor-dependent pathway in the liver utilizes the lysosomal route [45], although some apo B may use a more direct extra-lysosomal route [46]. It remains to be established if the more rapid delivery of cholesteryl esters from HDL into bile is mediated by the extralysosomal route, which may act as a 'higher-speed' pathway. Both LDL- and HDL-derived cholesteryl esters are secreted in the bile mainly in the form of bile acids. Thus, both have to follow the biosynthetic pathway of bile acids. The difference between LDL and HDL cholesteryl ester processing may therefore not be the conversion in bile acids itself but rather the rate of availability of the cholesterol moiety for bile-synthesizing enzymes in the cell. It is remarkable that, although the serum decay and liver association of [^3H]cholesteryl esters from HDL are hardly influenced by oestradiol treatment, a marked increase in the bile acid-secretion rate occurs during the first 24 h as compared with control rats. This is in contradiction with the increased acyl-CoA:cholesteryl acyltransferase activity [28,47] and the decreased 7α -hydroxylase activity [48,49] caused by oestradiol treatment. Further investigations on the mechanism of the selective uptake pathway of HDL cholesteryl esters may lead to a better understanding of oestradiol-induced changes.

Recently we showed that, *in vivo*, HDL can serve as an acceptor for cholesterol from liver endothelial cells and is necessary for an efficient delivery of cholesterol to parenchymal cells [50]. During circulation in the blood, [^3H]cholesterol was converted into [^3H]cholesteryl esters. In the present study we show that uptake of HDL cholesteryl esters in parenchymal cells is coupled to the rapid synthesis of bile acids, and thus we provide further evidence *in vivo* for the properties of HDL as an effective mediator in the cholesterol reversed-transport system.

This study was financially supported by the Dutch Foundation for Medical Research and Health Research (MEDIGON) grant no. 900-523-091. We thank Mrs. M. I. Wieriks for typing the manuscript.

REFERENCES

1. Glomset, J. A. (1968) *J. Lipid Res.* **9**, 155–167
2. Davis, R. A., Highsmith, W. E., Malone-McNeal, M., Archambault-Schexnayder, J. & Kuan, J.-C. W. (1983) *J. Biol. Chem.* **258**, 4079–4082
3. Fry, R. P., Mayes, P. A., Suckling, K. E. & Botham, K. M. (1990) *Biochim. Biophys. Acta* **1042**, 413–416

4. Ford, R. P., Botham, K. M., Suckling, K. E. & Boyd, G. S. (1985) *FEBS Lett.* **179**, 177–180
5. MacKinnon, A. M., Drevon, C. A., Sand, T. M. & Davis, R. A. (1987) *J. Lipid Res.* **28**, 847–855
6. Junker, L. H. & Davis, R. A. (1989) *J. Lipid Res.* **30**, 1933–1941
7. Whiting, M. J., Wishart, R. A., Lewis, G. & MacKinnon, A. M. (1989) *Biochim. Biophys. Acta* **1005**, 137–142
8. Herscovitz, H., Ronen, I., Bilu, S. & Tietz, A. (1986) *Biochim. Biophys. Acta* **878**, 426–434
9. Schwartz, C. C., Halloran, L. G., Vlahcevic, Z. R., Gregory, D. H. & Swell, L. (1978) *Science* **200**, 62–64
10. Bravo, E. & Cantafora, A. (1990) *Biochim. Biophys. Acta* **1045**, 74–80
11. Barter, P. J. & Lally, J. F. (1978) *Biochim. Biophys. Acta* **531**, 233–236
12. Redgrave, T. G., Roberts, D. C. K. & West, C. E. (1975) *Anal. Biochem.* **65**, 42–49
13. Pittman, R. C., Knecht, T. P., Rosenbaum, M. S. & Taylor, C. A., Jr. (1987) *J. Biol. Chem.* **262**, 2443–2450
14. Weisgraber, K. H. & Mahley, R. W. (1980) *J. Lipid Res.* **21**, 316–325
15. Schouten, D., Kleinherenbrink-Stins, M. F., Brouwer, A., Knook, D. L. & Van Berkel, Th. J. C. (1988) *Biochem. J.* **256**, 615–621
16. McFarlane, A. S. (1958) *Nature (London)* **182**, 53–57
17. Bilheimer, D. W., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **260**, 212–221
18. Van Berkel, Th. J. C., Kruijt, J. K. & Kempen, H. J. M. (1985) *J. Biol. Chem.* **260**, 12202–12207
19. Nagelkerke, J. F., Barto, K. P. & Van Berkel, Th. J. C. (1983) *J. Biol. Chem.* **258**, 12221–12227
20. Kuipers, F., Havinga, R., Bosschieter, H., Toorop, G. P., Hindriks, F. R. & Vonk, R. J. (1985) *Gastroenterology* **88**, 402–411
21. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
23. Glass, C., Pittman, R. C., Weinstein, D. B., Steinberg, D. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5435–5439
24. Glass, C., Pittman, R. C., Civen, M. & Steinberg, D. (1985) *J. Biol. Chem.* **260**, 744–750
25. Leitersdorf, E., Stein, O., Eisenberg, S. & Stein, Y. (1984) *Biochim. Biophys. Acta* **796**, 72–82
26. Harkes, L. & Van Berkel, Th. J. C. (1984) *Biochem. J.* **224**, 21–27
27. Pittman, T. C., Attie, A. D., Carew, T. E. & Steinberg, D. (1982) *Biochim. Biophys. Acta* **710**, 7–14
28. Nagelkerke, J. F., Bakkeren, H. F., Kuipers, F., Vonk, R. J. & Van Berkel, Th. J. C. (1986) *J. Biol. Chem.* **261**, 8908–8913
29. Kuipers, F., Nagelkerke, J. F., Bakkeren, H. F., Havinga, R., Van Berkel, Th. J. C. & Vonk, R. J. (1989) *Biochem. J.* **257**, 699–704
30. Harkes, L. & Van Berkel, Th. J. C. (1983) *FEBS Lett.* **154**, 75–80
31. Leitersdorf, E., Israeli, A., Stein, O., Eisenberg, S. & Stein, Y. (1986) *Biochim. Biophys. Acta* **878**, 320–329
32. Arbeeny, C. M., Rifci, V. A. & Eder, H. A. (1987) *Biochim. Biophys. Acta* **917**, 9–17
33. Wishart, R. & MacKinnon, M. (1990) *Biochim. Biophys. Acta* **1044**, 375–381
34. Rinniger, F. & Pittman, R. C. (1987) *J. Lipid Res.* **28**, 1313–1325
35. Gwynne, J. T. & Mahaffee, D. D. (1989) *J. Biol. Chem.* **264**, 8141–8150
36. Rinniger, F. & Pittman, R. C. (1988) *J. Lipid Res.* **29**, 1179–1194
37. Ose, L., Ose, T., Norum, K. R. & Berg, T. (1979) *Biochim. Biophys. Acta* **574**, 521–536
38. Van Berkel, Th. J. C., Kruijt, J. K., Van Gent, T. & Van Tol, A. (1980) *Biochem. Biophys. Res. Commun.* **92**, 1002–1008
39. Bachorik, P. S., Franklin, F. A., Virgil, D. G. & Kwiterovich, P. O., Jr. (1982) *Biochemistry* **21**, 5675–5684
40. Ose, L., Roken, I., Norum, K. R., Drevon, C. H. & Berg, T. (1981) *Scand. J. Clin. Lab. Invest.* **41**, 63–73
41. Wandel, M., Norum, K. R., Berg, T. & Ose, L. (1981) *Scand. J. Gastroenterol.* **16**, 71–80
42. Rifci, V. A. & Eder, H. A. (1984) *J. Biol. Chem.* **259**, 13814–13818
43. Schouten, D., Kleinherenbrink-Stins, M. F., Brouwer, A., Knook, D. L., Kamps, J. A. A. M., Kuiper, J. & Van Berkel, Th. J. C. (1990) *Arteriosclerosis* **10**, 1127–1135
44. Miller, N. E., La Ville, A. & Crook, D. (1985) *Nature (London)* **313**, 109–111
45. Chao, Y., Jones, A. L., Hradek, G. T., Windler, E. E. T. & Havel, R. J. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 597–601
46. Kleinherenbrink-Stins, M. F., Van der Boom, J., Bakkeren, H. F., Roholl, P. J. M., Brouwer, A., Van Berkel, Th. J. C. & Knook, D. L. (1990) *Lab. Invest.* **63**, 73–86
47. Davis, R. A., Showaller, R. & Kern, F., Jr. (1978) *Biochem. J.* **174**, 45–51
48. Bonorris, G. C., Coyne, M. J. & Chun, A. (1977) *J. Lab. Clin. Med.* **90**, 963–970
49. Davis, R. A., Elliot, T. S. & Lattier, G. A. (1986) *Biochemistry* **25**, 1623–1636
50. Bakkeren, H. F., Kuipers, F., Vonk, R. J. & Van Berkel, Th. J. C. (1990) *Biochem. J.* **268**, 685–691

Received 17 May 1991/16 July 1991; accepted 18 July 1991