THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1985 by The American Society of Biological Chemists, Inc.

Vol. 260, No. 22, Issue of October 5, pp. 12203-12207, 1985
Printed in U.S.A.

Specific Targeting of High Density Lipoproteins to Liver Hepatocytes by Incorporation of a Tris-Galactoside-terminated Cholesterol Derivative*

(Received for publication, March 19, 1985)

Theo J. C. van Berkel‡§, J. Kar Kruijt‡, and Herman-Jan M. Kempen¶

From the ‡Department of Biochemistry I, Erasmus University Rotterdam, 3000 DR Rotterdam and the ¶Gaubius Institute, Health Research Division TNO, Herenstraat 5d, 2313 AD Leiden, The Netherlands

A triantennary galactose-terminated cholesterol derivative, *N*-(tris(β-D-galactopyranosyloxymethyl) methyl)-N^{α}-(4(5-cholesten-3β-yloxy)succinyl)glycinamide (Tris-Gal-Chol), which dissolves easily in water, was added to human apolipoprotein E-free high density lipoproteins (HDL) in varying quantities. Incorporation of 5 or 13 μg of Tris-Gal-Chol into HDL (20 μg of protein) stimulates the liver association of the HDL apoprotein radioactivity 24- and 55-fold, respectively, at 10 min after intravenous injection into rats. The increased interaction of Tris-Gal-Chol HDL with the liver is blocked by preinjection of asialofetuin or *N*-acetylgalactosamine but not influenced by *N*-acetylglucosamine. The parenchymal liver cell uptake of HDL is stimulated 42- or 105-fold, respectively, by incorporation of 5 or 13 μg of Tris-Gal-Chol into HDL (20 μg of protein), while the association with nonparenchymal cells is stimulated only 1.7- or 5-fold. It can be calculated that 98.0% of the Tris-Gal-Chol HDL is associated with parenchymal cells. In contrast, incorporation of 13 μg of Tris-Gal-Chol into LDL (20 μg of protein) leads to a selective association of LDL with nonparenchymal cells (92.3% of the total liver uptake). It is concluded that Tris-Gal-Chol incorporation into HDL leads to a specific interaction of HDL with the asialoglycoprotein (galactose) receptor on parenchymal cells whereas Tris-Gal-Chol incorporation into LDL leads mainly to an interaction with a galactose receptor from Kupffer cells. Probably this highly selective cellular targeting of LDL and HDL by Tris-Gal-Chol is caused by the difference in size between these lipoproteins.

The increased interaction of HDL with the parenchymal cells upon Tris-Gal-Chol incorporation is followed by degradation of the apolipoprotein in the lysosomes.

It is concluded that Tris-Gal-Chol incorporation into LDL or HDL leads to a markedly increased catabolism of LDL by way of the Kupffer cells and HDL by parenchymal cells which might be used for lowering serum cholesterol levels. The use of Tris-Gal-Chol might also find application for targeting drugs or other compounds of interest to either Kupffer or parenchymal liver cells.

High plasma levels of low density lipoprotein (LDL[1]) cholesterol are correlated with an increased occurrence of atherosclerosis (1, 2). The liver is the only organ where cholesterol can be removed irreversibly from the circulation (3). Current treatments intend to increase the number of LDL receptors in liver. This is achieved by administration of a bile acid sequestrant, preferably in combination with an inhibitor of cholesterol synthesis (4). Recently we described (5) that the interaction of LDL with liver receptors can be markedly increased by mixing LDL with a triantennary galactose-terminated cholesterol derivative (Tris-Gal$_3$Chol). The Tris-Gal-Chol molecule was synthesized for use in directing LDL to the liver parenchymal cells because it was anticipated that the galactose residue should allow an interaction with the classical asialoglycoprotein (galactose) receptor on hepatocytes (6, 7). Surprisingly it was found that Tris-Gal-Chol incorporation into LDL or liposomes induces recognition of these particles by galactose receptors on nonparenchymal cells, and 80–90% of the Tris-Gal-Chol-induced liver uptake of LDL was exerted by these cells (8).

Upon addition to whole human plasma, Tris-Gal-Chol was found to become rapidly associated not only with LDL but also with HDL (5). The present paper describes the effect of Tris-Gal-Chol loading of HDL on the kinetics of clearance from the blood, the role of the liver herein, and the characteristics of the recognition site. Furthermore, the relative importance of the different liver cells in the Tris-Gal-Chol-induced uptake of HDL and the intracellular sites involved in HDL processing are determined. It is anticipated that the presented knowledge concerning the specific effects of Tris-Gal-Chol on the fate of the lipoproteins is necessary before clinical trials in hypercholesterolemic patients are justified.

MATERIALS AND METHODS

Chemicals—Metrizamide was obtained from Neyegaard & Co., A/S, Oslo, Norway. Collagenase type I, albumin (fraction V, defatted), chloroquine, cholesterol, *N*-acetyl-D-galactosamine and *N*-acetyl-D-glucosamine were purchased from Sigma. ^{125}I and ^{131}I (carrier free) in NaOH were purchased from New England Nuclear Chemicals, Dreieich, Germany, Ham's F-10 medium from Gibco-Europe, Hoofddorp, The Netherlands, and leupeptin from Peptide Institute Inc., Osaka, Japan.

Synthesis of Tris-Gal-Chol—Synthesis and characterization of the triantennary galactose-terminated cholesterol derivative are described in detail elsewhere (5).

HDL, LDL, Isolation, Iodination, and Tris-Gal-Chol Incorporation—HDL was isolated from human plasma at density $1.063 < d <$

* This research was partly supported by Grant 31.014 from the Dutch Heart Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Established Investigator of the Dutch Heart Foundation. To whom correspondence should be addressed: Department of Biochemistry I, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

[1] The abbreviations used are: LDL, low density lipoproteins; HDL, high density lipoproteins; Tris-Gal-Chol, *N*-(tris(β-D-galactopyranosyloxymethyl)methyl-N^{α}-(4-(5-cholesten-3β-yloxy)succinyl)glycinamide; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

1.21 by centrifugation according to Ref. 9. The HDL was passed over a Sepharose-heparin column (10), and the apo-E-free fraction was used throughout this study. LDL was isolated from human plasma at density $1.024 < d < 1.055$ g/ml by two repetitive centrifugations according to Ref. 9 as previously described (8). Radioiodination of HDL and LDL was done according to a modification (11) of the ICl method described in Ref. 12. Further characteristics of this LDL preparation were as described (8).

Tris-Gal-Chol was incorporated into HDL or LDL by mixing various amounts of Tris-Gal-Chol (from a stock solution in a final volume of 1 mg/1.5 ml phosphate-buffered saline) with 20 μg of iodinated HDL or LDL protein. After 10 min at room temperature the mixture was used for the described experiments. After incubation of 20 μg of LDL or HDL with 13 μg of Tris-Gal-Chol, analysis by density gradient centrifugation (9) indicated that the peak density had shifted from 1.040 to 1.059 (LDL) or from 1.114 to 1.132 (HDL). Both protein and cholesterol shifted in parallel, indicating that the particles remained intact with this degree of loading. Furthermore, all Tris-Gal-Chol was recovered in the same density fractions as the lipoproteins. However, when the lipoproteins were incubated with a 10-fold excess of Tris-Gal-Chol, they were disintegrated into various particles containing cholesterol, protein, and Tris-Gal-Chol in different proportions (not shown).

In Vivo Serum Clearance and Liver Association—Throughout this study 12-week-old male Wistar rats were used. Rats were anesthetized by intraperitoneal injection of 20 mg of Nembutal. The abdomen was opened, and radiolabeled compounds were injected into the inferior vena cava at the level of the renal veins. The body temperature of the rats was maintained at 36.5–37 °C by an infrared heating lamp monitored as the rectal temperature. At the indicated times 0.2 ml of blood was taken from the inferior vena cava at least 2 cm distal of the injection point. The samples were centrifuged for 2 min at 20,000 × *g*, and the radioactivity in the supernatants was counted. Liver lobules were tied off and excised at the indicated times. After weighing the lobule and counting its radioactivity, the total liver uptake was calculated using the assumption that 3.75% of the total body weight is contributed by the liver (13). The amount of liver that was tied off was 2–3% at each time point, so that at the longest circulation time still less than 10% of the total liver weight was removed. For the experiments reported in Figs. 1, 2, and 5 the liver was not perfused and the percentual liver value includes the amount of sample present in the entrapped blood (approximately 9% of the serum value based upon [^{3}H]albumin measurements). To indicate the maximal contribution of the entrapped blood to the liver value a *dotted line* is drawn in the relevant figures. Agents tested for their effect on the lipoprotein uptake by the liver were injected into rats as a bolus at the indicated time prior to the radiolabeled compound.

Cell Isolation Procedures—Rats were anesthetized and injected with the radiolabeled compounds in a similar way as for the determination of the total liver uptake. At 10 min after injection the vena porta was cannulated, and a liver perfusion was started with Hanks' buffer, pH 7.4, plus Hepes (2.4 g/liter) at 8 °C. After 8-min perfusion (flow rate, 14 ml/min) a lobule was tied off for determination of the total liver uptake. In order to separate the various cell types the liver was subsequently subjected to a low temperature (8 °C) perfusion with inclusion of 0.05% w/v collagenase. The separation of parenchymal and nonparenchymal cells was performed at 8 °C as described (8).

When indicated, a further subdivision of the nonparenchymal cell preparation into endothelial cells and Kupffer cells was performed by centrifugal elutriation exactly as described earlier (14). Calculation of the contribution of the different cell types to total liver uptake was performed as described (14). As found earlier (8, 14) with other substrates, no loss of cell-bound or formation of acid-soluble radioactivity occurred during the low temperature cell isolation procedures, leading to a quantitative recovery of the radioactivity associated with total liver in the subsequently isolated cells. This was checked for each individual cell isolation by comparing the calculated (from the relative contribution of the various cell types) and determined total liver association.

Subcellular Fractionation—Fractionation of total liver was performed by differential centrifugation exactly as described (15).

Assay of Enzyme Activities—Acid phosphatase activity was determined according to Ref. 16, β-glucuronidase according to Ref. 17, and cathepsin D as described in Ref. 18.

RESULTS

Serum Decay and Liver Association of Tris-Gal-Chol HDL—The disappearance of apo-E-free HDL from serum proceeds at a low rate, and only 1.0 ± 0.1% ($n = 4$) of the iodine radioactivity is found in liver at 10 min after injection (values obtained after liver perfusion). Incorporation of different amounts of Tris-Gal-Chol into HDL leads to a dose-dependent increased removal from serum with a quantitative recovery of the label in liver (Fig. 1). The liver association of Tris-Gal-Chol HDL is very rapid (nearly maximal at 2 min after injection) while between 15 and 30 min after injection a clear decrease in liver-associated radioactivity is noticed without any change in serum radioactivity. Earlier we found that a low degree of loading of LDL with Tris-Gal-Chol leads to a reversible liver association because reappearance of trichloroacetic acid-precipitable radioactivity in serum occurs (8). With HDL such a reversible association is not observed (Fig. 1).

The increased serum decay and liver association of Tris-Gal-Chol HDL is completely blocked by preinjection (1 min before Tris-Gal-Chol HDL) of 0.5 mmol/rat *N*-acetylgalactosamine (GalNAc, final concentration in the blood approximately 50 mM), while preinjection of *N*-acetylglucosamine (GlcNAc) has hardly any effect. Preinjection of 5 or 25 mg of asialofetuin/rat leads to a complete blockade of the increased liver association of Tris-Gal-Chol HDL, similar as with GalNAc (Fig. 2).

Cellular Distribution of Tris-Gal-Chol HDL in Liver—The complete inhibition of the Tris-Gal-Chol-induced liver uptake of HDL by asialofetuin suggests that the asialoglycoprotein receptor on parenchymal cells is responsible for this uptake. At 10 min after injection the HDL association with parenchymal cells (2.8 ± 0.1% of the injected dose × 10^4/mg cell protein, $n = 4$) is stimulated 42- or 105-fold, respectively, by incorporation of 5 or 13 μg of Tris-Gal-Chol/20 μg of HDL apoprotein (Fig. 3). For nonparenchymal cells the association of HDL (17.5 ± 1.1% of the injected dose × 10^4/mg of cell protein, $n = 4$) is stimulated 1.7- or 5-fold, respectively, by 5 or 13 μg of Tris-Gal-Chol incorporated into HDL. When 200 μg of Tris-Gal-Chol is mixed with 20 μg of HDL (loading to its disintegration) especially the nonparenchymal cell uptake shows a further increase (Fig. 3).

The capacity of the liver uptake system for Tris-Gal-Chol-loaded HDL was studied by injection of 100 and 250 μg of HDL (mixed with Tris-Gal-Chol in the ratio HDL protein:Tris-Gal-Chol of 20:13). Both with 100 and 250 μg of Tris-Gal-Chol HDL the total liver uptake (in per cent of the injected dose) was reduced by about 50% as compared with 20 μg of Tris-Gal-Chol HDL. The percentage of the injected dose taken up by the parenchymal cells was about two times lower than with the low dose of 20 μg of Tris-Gal-Chol HDL, while the percentage uptake by the nonparenchymal cells was not changed.

Since the cellular distribution of Tris-Gal-Chol HDL was seen to be so different from that of Tris-Gal-Chol-loaded LDL (8), we decided to compare the cellular uptakes of the Tris-Gal-Chol-loaded lipoproteins, as well as of asialofetuin, directly in one set of experiments. Furthermore, we specified the cellular uptake sites more precisely by separating endothelial from Kupffer cells (14). As indicated in Fig. 4, Tris-Gal-Chol stimulates the total liver uptake of LDL or HDL to a similar extent. However, the parenchymal cell uptake of Tris-Gal-Chol HDL is much higher than that of Tris-Gal-Chol LDL, whereas the reverse is seen with the Kupffer cells. Both for Kupffer and for endothelial cells, Tris-Gal-Chol incorporation stimulated the uptake of LDL but had only a

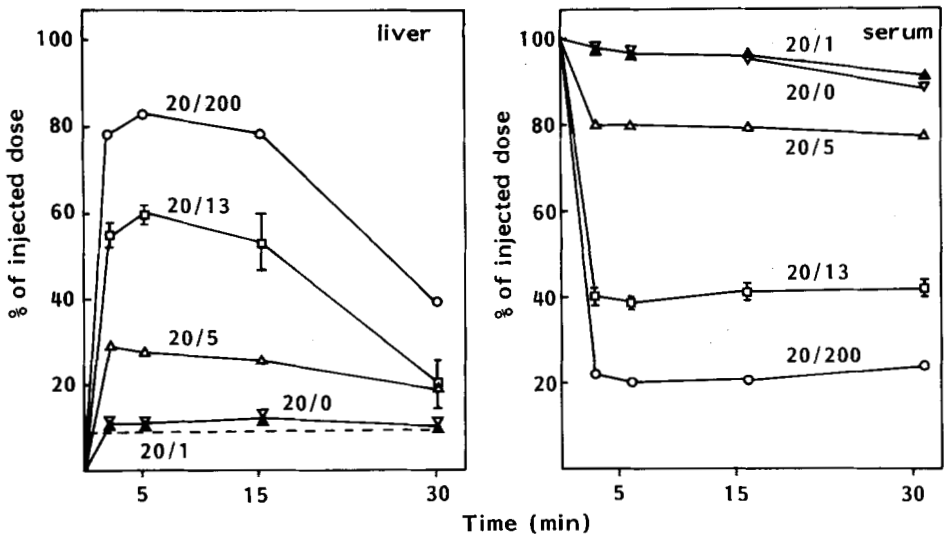

FIG. 1. **Effect of Tris-Gal-Chol on the liver association and serum decay of HDL.** ^{125}I-HDL (20 μg of apolipoprotein) was mixed with 0 (▽), 1 (▲), 5 (△), 13 (□), or 200 μg of Tris-Gal-Chol (○). The mixture was injected into anesthetized rats, and the liver association and serum decay was determined. When indicated, *bars* represent S.E. for 3 animals. The livers were not perfused, and the *dotted line* represents the maximal contribution of the serum value to the liver uptake (determined with [^{3}H]albumin).

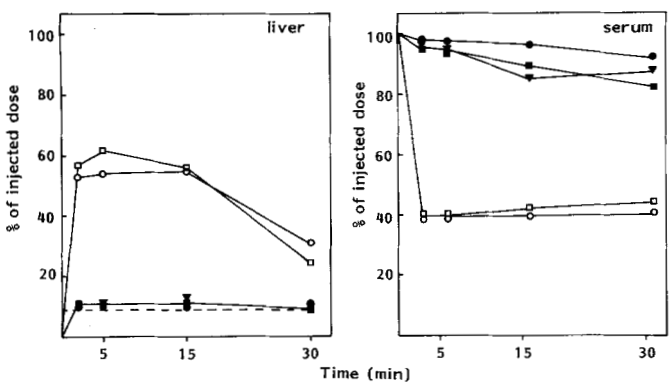

FIG. 2. **Effect of asialofetuin, GalNAc, and GlcNAc on the liver association and serum decay of Tris-Gal-Chol HDL.** ^{125}I-HDL (20 μg of apolipoprotein) was mixed with 13 μg of Tris-Gal-Chol. One min prior to injection of Tris-Gal-Chol HDL, either solvent (□), 5 mg (▼) or 25 mg of asialofetuin (■), 0.5 mmol of GalNAc (●), or 0.5 mmol of GlcNAc (○) was preinjected. Further conditions as in Fig. 1.

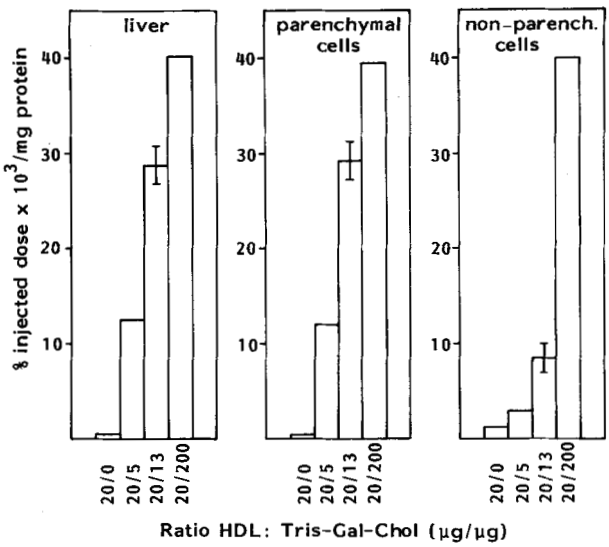

FIG. 3. **The effect of Tris-Gal-Chol on the association of HDL to parenchymal and nonparenchymal cells *in vivo*.** ^{125}I-HDL (20 μg of apolipoprotein) was mixed with 0 (20/0), 5 (20/5), 13 (20/13), or 200 (20/200) μg of Tris-Gal-Chol. Ten minutes after injection a liver perfusion was started and the total liver association (after 8 min of perfusion at 8 °C) and the association to the subsequently isolated (at 8 °C) parenchymal and nonparenchymal cells was determined. The *bars* represent values ±S.E. (n = 3).

small effect on that of HDL. Asialofetuin follows a similar cellular uptake pattern as Tris-Gal-Chol HDL although the cell association of Tris-Gal-Chol HDL to endothelial and Kupffer cells is even lower than that of asialofetuin.

From these data it can be calculated, taking into account the amount of protein contributed by each cell type to the total liver, for which percentage the various liver cell types are responsible for the total liver uptake of asialofetuin and Tris-Gal-Chol-loaded lipoproteins (Table I).

Processing of Tris-Gal-Chol HDL—The intracellular processing of Tris-Gal-Chol HDL was investigated by isolating hepatocytes 10 min after the *in vivo* injection of Tris-Gal-Chol HDL (20/13) and incubating the cells at 37 °C *in vitro*. Within 30 min of incubation the cells lose 45 ± 1% (n = 2) of their radioactivity, of which 79 ± 1% is recovered as trichloroacetic acid soluble in the cell supernatant.

The possible involvement of the lysosomes in processing Tris-Gal-Chol HDL was investigated by pretreating the rats with either leupeptin (19) or chloroquine (20) and following the disappearance of label from liver (Fig. 5). Pretreatment of the rats with either leupeptin or chloroquine clearly inhibits the processing of Tris-Gal-Chol HDL. The serum radioactivities were not influenced by the various treatments (not shown). At 30 min after injection of Tris-Gal-Chol-loaded ^{125}I-HDL in rats pretreated with leupeptin or chloroquine a subcellular distribution study was performed (Fig. 6, *A* and *B*). It appears that the radioactivity is highly enriched in the lysosomal fraction. Chloroquine pretreatment also leads to accumulation of radioactivity in the microsomal fraction. In untreated liver the lysosomal marker acid phosphatase shows a high relative activity in the L fraction (8, 15). However, the pretreatment of the rats with leupeptin or chloroquine causes a shift in its distribution. A similar shift was found for the lysosomal markers β-glucuronidase and cathepsin D (data not shown).

DISCUSSION

The Tris-Gal-Chol molecule was designed for use in directing lipoproteins to the liver parenchymal cells because it was anticipated that the galactose residues should allow an interaction with the asialoglycoprotein (galactose) receptor on hepatocytes (6, 7). The design of the compound was also adapted to the property of the hepatocyte receptor to bind

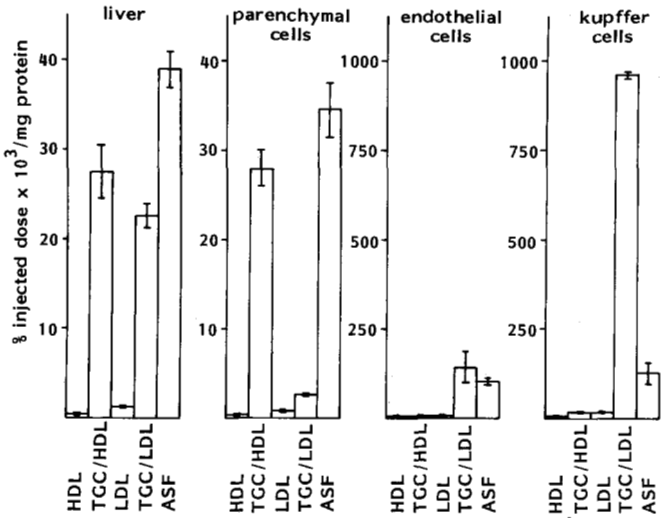

FIG. 4. **The effect of Tris-Gal-Chol on the association of HDL and LDL to parenchymal, endothelial, and Kupffer cells in comparison with the association of asialofetuin (*ASF*).** ^{125}I-HDL or ^{125}I-LDL (20 μg of apolipoprotein) were mixed with 13 μg of Tris-Gal-Chol (*TGC/HDL* or *TGC/LDL*) or the equivalent amount of phosphate-buffered saline (HDL or LDL). Ten minutes after injection of the apolipoproteins or ^{125}I-asialofetuin (9 μg), a liver perfusion was started, and the total liver association (after an 8-min perfusion at 8 °C) and the association with the subsequently isolated (at 8 °C) parenchymal, endothelial, and Kupffer cells was determined. The *bars* represent values ±S.E. (n = 3).

TABLE I

Relative contribution of the different liver cell types to the total liver uptake of asialofetuin, Tris-Gal-Chol HDL, and Tris-Gal-Chol LDL

The amount of radioactivity/mg cell protein in the isolated cell fractions was multiplied with the amount of protein that each cell type contributes to total liver protein. Lipoproteins (20 μg of apolipoproteins) were mixed with 13 μg of Tris-Gal-Chol. The values are calculated from the mean of 3 independent experiments for each substrate.

Cell type	Asialofetuin	Tris-Gal-Chol HDL	Tris-Gal-Chol LDL
Parenchymal cells (%)	82.5	98.0	7.7
Endothelial cells (%)	9.3	0.5	15.5
Kupffer cells (%)	8.2	1.5	76.8

and internalize triantennary structures with a much higher affinity and efficiency than bis- or monoglycosides (21, 22). Furthermore Attie and co-workers (23) reported that lactosylated LDL was effectively catabolized by the asialoglycoprotein receptor from rat hepatocytes in culture. Earlier we showed (8) that incorporation of Tris-Gal-Chol in LDL leads to an increased galactose-mediated interaction of LDL with the liver and that the nonparenchymal cells are responsible by 80–90% for the increased uptake. The present data confirm this conclusion and give evidence that within the nonparenchymal cells especially the Kupffer cells show a high affinity for Tris-Gal-Chol LDL. Incorporation of the Tris-Gal-Chol molecule into HDL leads to a similar dose-dependent increase in interaction with the liver as with LDL, but now surprisingly 98% of the Tris-Gal-Chol HDL is taken up by parenchymal cells. The association of Tris-Gal-Chol HDL to total liver is blocked by asialofetuin (5 or 25 mg) and by GalNAc but not influenced by GlcNAc, so it can be concluded that Tris-Gal-Chol incorporation into HDL leads to a specific interaction of HDL with the asialoglycoprotein (galactose) receptor on parenchymal cells. Actually this interaction is more specific

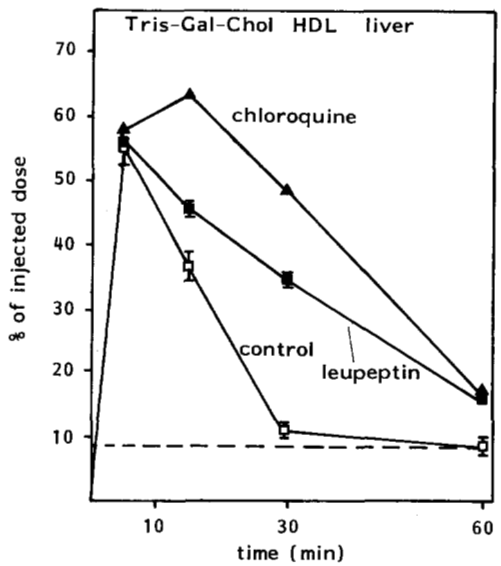

FIG. 5. **The effect of leupeptin and chloroquine on the liver association of Tris-Gal-Chol HDL.** ^{125}I-HDL (20 μg of apolipoprotein) was mixed with 13 μg of Tris-Gal-Chol. The mixtures were injected into rats which were preinjected with 5 mg of leupeptin (60 min prior to the lipoproteins) or with chloroquine (120 and 60 min prior to injection of the lipoproteins). The control was preinjected at 60 min prior to the lipoproteins with phosphate-buffered saline. The *bars* represent values ±S.E. (n = 3). Further conditions are as described in the legend to Fig. 1.

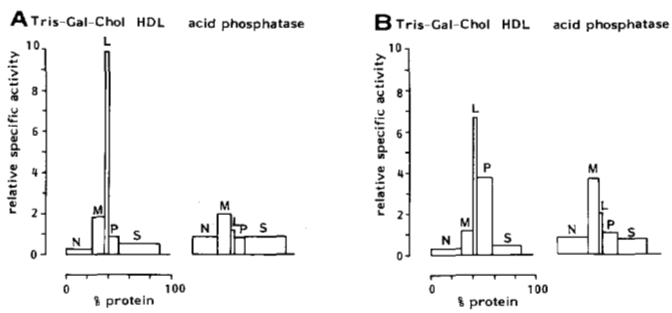

FIG. 6. **Distribution of Tris-Gal-Chol HDL in subcellular fractions of the liver.** ^{125}I-HDL (20 μg of apolipoprotein) was mixed with 13 μg of Tris-Gal-Chol. The mixture was injected into rats which were preinjected with 5 mg of leupeptin (60 min prior to injection of Tris-Gal-Chol HDL (*A*)) or with chloroquine (120 and 60 min prior to injection of Tris-Gal-Chol HDL (*B*)). 30 minutes after injection of Tris-Gal-Chol HDL a subcellular distribution procedure was started exactly as described in Ref. 15. *N* is nuclear fraction, *M* is heavy mitochondrial fraction, *L* is light mitochondrial fraction, *P* is microsomal fraction, and *S* is the final supernatant.

for Tris-Gal-Chol HDL than for asialofetuin itself, because in accordance with autoradiographic data (7, 24) some asialofetuin (10–20%) of the total liver uptake becomes associated with Kupffer and endothelial cells.[2]

The molecular mechanism by which association of Tris-Gal-Chol with HDL or LDL leads to such a differential targeting to galactose receptors on hepatocytes or Kupffer cells, respectively, may be related to the ultrastructural receptor characteristics. The galactose receptor on hepatocytes is

[2] In accordance with the autoradiographic data of Hubbard *et al.* (7) we found that preinjection of 5 and 25 mg of asialofetuin inhibited the association of ^{125}I-asialofetuin to parenchymal cells by 84.2 and 92.6%, respectively, while the association to endothelial and Kupffer cells was only inhibited by 22.3 and 28.5%, respectively (*cf.* Hubbard *et al.* (7) with 20 mg of asialofetuin for parenchymal cells 99% and endothelial and Kupffer cells 24% inhibition).

randomly distributed on the capillary side of the plasma membrane, while the Kupffer cell receptors are preclustered in coated pits (25, 26). When asialofetuin is coated to gold particles of 5 nm an interaction with both hepatocytes and Kupffer cells was reported (27) while coating on 17-nm gold particles prevented the interaction with hepatocytes. Since HDL possesses a mean size of 10 nm and LDL of 23 nm (28), the size of the lipoprotein might be responsible for the differential fate of the Tris-Gal-Chol-loaded particles irrespective of the fact that an identical recognition marker is present. If lipoprotein receptors on various cell types should possess similar properties as the galactose receptors of liver, this might have important implications for lipoprotein metabolism because for instance apolipoprotein E might be recognized by different cells when present on lipoproteins of various sizes. In addition to the hypothesis with respect to the size of the particles as explanation for the different fate of LDL and HDL, it is possible that different apoproteins may differently influence the orientation of Tris-Gal-Chol in the particle and thus the interaction with randomly and preclustered galactose receptors.

The hepatic processing of Tris-Gal-Chol HDL appears to involve the lysosomal compartment. The decrease in total hepatic radioactivity at the longer times after injection of Tris-Gal-Chol HDL is clearly inhibited by pretreatment of the rats with leupeptin or chloroquine. Furthermore, in the animals pretreated with the lysosomotropic agents, HDL apolipoprotein radioactivity accumulates in the lysosomal fraction at 30 min after injection. Leupeptin is an inhibitor of thiol proteases (29), while chloroquine acts as a general inhibitor of lysosomal proteolysis by increasing the lysosomal pH (30). In addition chloroquine may inhibit the fusion of endocytotic vesicles or multivesicular bodies with lysosomes (20, 31). Both leupeptin and chloroquine treatments lead to the appearance of prominent autolysosomes with a concurrent change in the density profile of acid phosphatase (32). We found a similar shift with β-glucuronidase or cathepsin D. The accumulation of ^{125}I-HDL radioactivity in the fraction which does contain the highest relative activity of lysosomal enzymes in untreated rats (18) indicates that the newly formed endocytotic vesicles and/or multivesicular bodies do not fuse with pre-existing autolysosomes. The relatively higher amount of radioactivity in the microsomal fraction after chloroquine treatment as compared to leupeptin might be related to the additional inhibitory action of chloroquine on the fusion of endocytic vesicles or multivesicular bodies with lysosomes (20, 32) as these vesicles are expected in this fraction (33).

What are the consequences of our findings in view of a potential future application of Tris-Gal-Chol as a hypocholesterolemic drug? Upon addition of Tris-Gal-Chol to whole plasma, the compound becomes associated with both LDL and HDL, roughly in proportion to the available lipid surface (5). Therefore, upon intravenous administration of the compound the removal of both lipoproteins can be accelerated, LDL by way of the Kupffer cells, HDL via the parenchymal cells of the liver. Although low serum levels of HDL are associated with increased risk of atherosclerotic disease (34), it is at present uncertain whether enhanced hepatic removal of HDL would be unfavorable. If so, this could possibly be slowed down by co-administration of asialoglycoproteins, competing for the same receptor on the parenchymal cells.

The use of Tris-Gal-Chol might also find application in targeting drugs, hormones, or other material of interest to specific liver cell types. The use of liposomes as transport vesicles is hampered by the difficulty to target these vesicles rapidly to parenchymal liver cells. The use of galactose residue-exposing liposomes is frustrated because these particles are mainly captured by the galactose receptor on Kupffer cells (35). The incorporation of Tris-Gal-Chol into HDL leads to a successful and rapid targeting to the asialoglycoprotein (galactose) receptor on hepatocytes. This property might be used to deliver any compound either covalently linked to the protein moiety or incorporated into the lipid core of HDL. The ability to introduce rapidly and selectively acetylated LDL into endothelial liver cells (14, 36, 37), Tris-Gal-Chol LDL into Kupffer cells, and Tris-Gal-Chol HDL into parenchymal cells will also help in unraveling the complex metabolic interaction between the various liver cell types.

Acknowledgment—Martha Wieriks is thanked for the preparation of the manuscript and secretarial help.

REFERENCES

1. Wilson, P. W., Garrison, R. J., Castelli, W. P., Feinleib, M., McNamara, P. M., and Kannel, W. B. (1980) *Am. J. Cardiol.* **46,** 649–654
2. Goldstein, J. L., and Brown, M. S. (1977) *Metabolism* **26,** 1257–1275
3. Lindstedt, S. (1970) in *Proceeding on the Second International Symposium on Atherosclerosis* (Jones, R. J., ed) pp. 262–271, Springer Verlag, New York
4. Bilheimer, D. W., Grundy, S. M., Brown, M. S., and Goldstein, J. L. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 1194–1198
5. Kempen, H. J. M., Hoes, C., Van Boom, J. H., Spanjer, H. H., De Lange, J., Langendoen, A., and Van Berkel, Th. J. C. (1984) *J. Med. Chem.* **27,** 1306–1312
6. Ashwell, G., and Morell, A. G. (1974) *Adv. Enzymol.* **41,** 99–128
7. Hubbard, A. L., Wilson, G., Ashwell, G., and Stukenbrok, H. (1979) *J. Cell Biol.* **83,** 47–64
8 Van Berkel, Th. J. C., Kruijt, J. K., Spanjer, H. H., Nagelkerke, J. F., Harkes, L., and Kempen, H. J. M. (1985) *J. Biol. Chem.* **260,** 2694–2699
9. Redgrave, T. G., Roberts, D. C. K., and West, C. E. (1975) *Anal. Biochem.* **65,** 42–49
10. Weisgraber, K. H., and Mahley, R. W. (1980) *J. Lipid. Res.* **21,** 316–325
11. Bilheimer, D. W., Eisenberg, S., and Levy, R. I. (1972) *Biochim. Biophys. Acta* **260,** 212–221
12. Mc Farlane, A. S. (1958) *Nature* **182,** 53
13. Van Berkel, Th. J. C., and Van Tol, A. (1978) *Biochim. Biophys. Acta* **530,** 299–304
14. Nagelkerke, J. F., Barto, K. P., and van Berkel, Th. J. C. (1983) *J. Biol. Chem.* **258,** 12221–12227
15. De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955) *Biochem. J.* **60,** 604–617
16. Bergmeyer, H. U. (1970) *Methoden der Enzymatischen Analyse,* pp. 457–458, Verlag Chemie, Weinheim, West Germany
17. Fishman, W. H., Springer, B., and Brunetti, R. (1948) *J. Biol. Chem.* **173,** 449–456
18. Gianetto, R., and De Duve, C. (1955) *Biochem. J.* **59,** 433–438
19. Dunn, W. A., LaBadie, J. H., and Aronson, N. N., Jr. (1979) *J. Biol. Chem.* **254,** 4191–4196
20. Hornick, C. A., Jones, A. L., Renaud, G., Hradek, G., and Havel, R. J. (1984) *Am. J. Physiol.* **246,** G187–G194
21. Lee, Y. C. (1978) *Carbohydr. Res.* **67,** 509–514
22. Kawaguchi, K., Kuhlenschmidt, M., Roseman, S., and Lee, Y. C. (1980) *Arch. Biochem. Biophys.* **205,** 388–395
23. Attie, A. D., Pittman, R. C., and Steinberg, D. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 5923–5927
24. Hubbard, A. L., and Stukenbrok, H. (1979) *J. Cell Biol.* **83,** 65–81
25. Kolb-Bachofen, V. (1981) *Biochim. Biophys. Acta* **645,** 293–299
26. Kolb-Bachofen, V., Schlepper-Schäfer, J., and Vogell, W. (1982) *Cell* **29,** 859–866
27. Roos, P. H., Kolb-Bachofen, V., Schlepper-Schäfer, J., Monsigny, M., Stockert, R. J., and Kolb, H. (1983) *FEBS Lett.* **157,** 253–256
28. Forte, T., and Nichols, A. V. (1972) *Adv. Lipid Res.* **10,** 1–41
29. Kirschke, H., Langner, J., Wiederanders, B., Ansorg, S., and Bohley, P. (1977) *Eur. J. Biochem.* **74,** 293–301
30. Ohkuma, S., and Poole, B. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 3327–3331
31. Tolleshaug, H., and Berg, T. (1979) *Biochem. Pharmacol.* **28,** 2919–2922
32. Furuno, K., Ishikawa, T., and Kato, K. (1982) *J. Biochem. (Tokyo)* **91,** 1485–1494
33. La Badie, J. H., Chapman, K. P., and Aronson, N. N. (1975) *Biochem. J.* **152,** 271–279
34. Miller, G. J., and Miller, N. E. (1975) *Lancet* 16–19
35. Spanjer, H. H., Morselt, H., and Scherphof, G. L. (1984) *Biochim. Biophys. Acta* **774,** 49–55
36. Blomhoff, R., Drevon, C. A., Eskild, W., Helgerud, P., Norum, K. R., and Berg, T. (1984) *J. Biol. Chem.* **259,** 8898–8903
37. Pitas, R. E., Boyles, J., Mahley, R. W., and Bissell, D. M. (1985) *J. Cell Biol.* **100,** 103–117