Turnover and uptake by organs of radioactive serum high-density lipoprotein cholesteryl esters and phospholipids in the rat *in vivo*

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The serum decay of rat serum high-density lipoprotein (HD lipoprotein), labelled bio-synthetically with ^{32}P in the phospholipid or with ^3H in the cholesteryl ester moiety, was measured in rats after partial hepatectomy or sham operation. The serum decay of ^3H -labelled HD lipoprotein cholesteryl esters was biexponential. In sham-operated rats the $t_{\frac{1}{2}}$ values for the rapid phase and the slow phase were 0.2 ± 0.1 h and 4.2 ± 0.4 h (means $\pm$ S.E.M.) respectively. After removal of two-thirds of the liver the $t_{\frac{1}{2}}$ value of the rapid phase did not change (0.1 ± 0.1 h), whereas the $t_{\frac{1}{2}}$ value of the slow phase increased to 5.7 ± 0.8 h. Partial hepatectomy hardly changed extrahepatic tissue radioactivities, whereas the percentage of the injected dose recovered in the liver 6 h after injection decreased from $34.0 \pm 1.9\%$ before to $13.5 \pm 1.6\%$ after partial hepatectomy. The ^{32}P -labelled HD lipoprotein phospholipids showed a rapid monoexponential decay from serum with $t_{\frac{1}{2}}$ values of 0.71 ± 0.3 h and 1.48 ± 0.11 h after sham operation or partial hepatectomy respectively. The tissue ^{32}P radioactivities in the sham-operated rats, measured 1 h after injection, were $46.0 \pm 1.7\%$ (liver), $1.7 \pm 0.3\%$ (adipose tissue), $3.7 \pm 1.2\%$ (skeletal muscle) and $3.0 \pm 0.0\%$ (erythrocytes) of the injected dose. Only the value for liver was affected by partial hepatectomy and decreased to $16.7 \pm 3.8\%$. In a previous publication [Van Tol, Van Gent, Van't Hooft & Vlasplolder (1978) *Atherosclerosis* 29, 439–448] we showed in a highly comparable experimental setting that the turnover rates of HD apolipoproteins A and C *in vivo* are not influenced by removal of two-thirds of the liver. From the present study it is clear that the removal rates of radioactive HD lipoprotein cholesteryl esters and HD lipoprotein phospholipids from serum *in vivo* are decreased by partial hepatectomy. The results indicate the possibility of partly separate metabolic pathways of HD apolipoproteins A and C, HD lipoprotein cholesteryl esters and HD lipoprotein phospholipids. The phospholipids and cholesteryl esters of HD lipoprotein are metabolized predominantly by the liver. Possible mechanisms for the hepatic uptake and metabolism of HD lipoprotein cholesteryl (esters) and phospholipids are discussed.

Low serum HD lipoprotein cholesteryl (ester) concentrations are associated with an increased prevalence of coronary heart disease, independently of serum triacylglycerol and LD lipoprotein cholesteryl (ester) concentration (Gordon *et al.*, 1977). It has been suggested by Glomset (1968) that serum

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein (density < 1.006 g/ml); ID lipoprotein, intermediate-density lipoprotein (density range 1.006–1.02 g/ml); LD lipoprotein, low-density lipoprotein (density range 1.02–1.05 g/ml); HD lipoprotein, high-density lipoprotein (density range 1.05–1.21 g/ml); F.T.R., fractional turnover rate.

HD lipoprotein plays a role in the transport of cholesterol from extrahepatic tissues (e.g. the arterial wall) to the liver. In this hypothesis tissue cholesterol is incorporated in HD lipoprotein as cholesteryl ester by the action of phosphatidylcholine:cholesterol acyltransferase and the lipoprotein particle is subsequently metabolized by the liver. From previous studies *in vivo* (Roheim *et al.*, 1971; Eisenberg *et al.*, 1973; Nakai & Whayne, 1976) it was concluded that the liver was an important site of removal of iodinated HD lipoprotein from the blood. It has also been shown that isolated liver cells are able to bind and degrade HD apolipoprotein and

cholesteryl esters (Nakai *et al.*, 1976; Drevon *et al.*, 1977; Van Berkel *et al.*, 1980). However, in a further study (Van Tol *et al.*, 1978a) we found no effect of partial hepatectomy on the fractional catabolic rate of HD apolipoproteins (A and C) *in vivo*. In addition, Sigurdsson *et al.* (1979) measured only about 7% of the catabolic rate of HD apolipoprotein *in vivo* using the isolated perfused rat liver.

From these investigations it seems questionable that the liver is the only organ responsible for the degradation of HD apolipoprotein *in vivo*. To assess the role of the liver in the metabolism of HD lipoprotein phospholipids and cholesteryl esters *in vivo*, we measured the serum decay of these biosynthetically labelled HD lipoprotein lipids in rats after partial hepatectomy or sham operation. The experimental conditions in the present study are highly comparable with the study on HD apolipoprotein catabolism described previously (Van Tol *et al.*, 1978a).

Materials and methods

Isolation and labelling of serum lipoproteins

Rat HD lipoprotein was labelled biosynthetically by injection of either $H_3^{32}PO_4$ or $[1\alpha,2\alpha(n)-^3H]$ cholesterol (complexed to bovine serum albumin) *in vivo* as described by Rubenstein & Rubenstein (1972). The radioactive materials were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Male Wistar rats, fed the standard laboratory chow and weighing 245–263 g, were used in all experiments. All values given are means $\pm$ S.E.M. for the indicated number of experiments.

HD lipoprotein was isolated from serum by density-gradient ultracentrifugation in the Beckman SW 40 rotor, by a modification of the method described by Redgrave *et al.* (1975), i.e. it was isolated from the density range 1.050–1.21 g/ml. For details see Van Tol *et al.* (1978a). The ^{32}P radioactivity in HD lipoprotein was mainly associated with phosphatidylcholine ($86.4 \pm 0.6\%$). Minor amounts were present in sphingomyelin ($9.6 \pm 0.7\%$), lysophosphatidylcholine ($2.6 \pm 0.8\%$) and phosphatidylethanolamine + phosphatidylserine ($1.5 \pm 0.1\%$). The HD lipoprotein, labelled biosynthetically in the cholesterol moiety as well as the cholesteryl ester moiety, was screened by injecting it intravenously into rats, to remove radioactive unesterified cholesterol. The recipient rats were exsanguinated 1 h later and the HD lipoprotein was isolated as described above; 91–95% of the 3H label of the screened preparation was associated with cholesteryl esters.

Procedures for (partial) hepatectomy, blood sampling and incubation of serum *in vitro*

Catheters were placed in the carotic artery and

the jugular vein, as described in detail previously (Van Tol *et al.*, 1978a). Partial hepatectomy, involving the removal of about two-thirds of the liver, or sham operation was performed directly after the cannulation procedure. Partial hepatectomy did not result in abnormal concentrations of serum glucose or total serum protein, as measured 8 h after the operation. The labelled HD lipoprotein (0.2 mg of protein) was injected into the jugular vein 2 h after the operations described above and blood samples of about 0.3 ml were obtained from the freely moving animals by way of the arterial cannula.

Functional evisceration was performed as described by Windler *et al.* (1980). The labelled HD lipoprotein was injected into a femoral vein, immediately after the last ligation of the evisceration procedure. The animals were bled from the abdominal aorta 30 min after injection.

For the experiments *in vitro*, serum was obtained as described in the following section. $[^3H]$ Cholesteryl ester-labelled HD lipoprotein was added to serum in such a way that the concentration of endogenous serum HD lipoprotein was increased by less than 5%. The mixture was incubated at 37°C for the indicated periods of time.

Analysis of serum decay curves, serum lipoproteins and tissue radioactivities

The first blood sample was obtained 3 min after injection of the labelled HD lipoprotein. At this time the injected radioactivity was quantitatively recovered in the serum (plasma volume 4.25 ml/100 g body wt.). The decay curves were analysed as described by Matthews (1957) and Langer *et al.* (1972).

At the end of the experiment the rats were sedated by injection of Nembutal (S. A. Abbott, Brussels, Belgium) and exsanguinated. The blood was collected and allowed to clot and the serum was isolated and subfractionated by density-gradient ultracentrifugation. For further details see Van Tol *et al.* (1978a).

After the animals were bled at the end of the decay experiments, total body perfusion was performed by way of the heart and the vena cava with at least 100 ml of saline at 38°C. This perfusion resulted in a virtually complete loss of blood from all tissues tested, except the spleen. Several samples of the small intestine, skeletal muscle, skin and adipose tissue were weighed and analysed for radioactivity. Skeletal muscle, adipose tissue, skin and erythrocytes were taken to be 45%, 10%, 18% and 4.25% of body wt. respectively (cf. Caster *et al.*, 1956). The weights of the other organs were measured for 10 animals and found to be $3.71 \pm 0.08\%$ (liver), $0.20 \pm 0.01\%$ (spleen), $0.34 \pm 0.01\%$ (heart), $0.77 \pm 0.02\%$ (kidneys), $0.49 \pm 0.02\%$ (lungs) and $3.2 \pm 0.1\%$ (small intestine).

For counting of ^{32}P radioactivity in organs 100–200 mg of tissue was digested in 1 ml of tissue solubilizer Soluene-350 at 50°C. After cooling to room temperature 0.2 ml of propan-2-ol and 0.2 ml of 30% H_2O_2 were added. After immediate shaking the solution was kept at 40°C for about 30 min before the addition of 10 ml of the scintillator Dimilume. Of the erythrocyte suspension 0.2 ml was mixed thoroughly with 1.5 ml of a Soluene-350/propan-2-ol (1:1, v/v) mixture. After addition of 0.5 ml of 30% H_2O_2 and immediate shaking, the solution was kept at room temperature for 10 min followed by incubation at 40°C for 10–15 min. After addition of 15 ml of 0.5 M-HCl/Instagel (1:9, v/v) mixture the samples were counted in a model 3380 Packard liquid-scintillation spectrometer.

For counting of ^3H radioactivity in organs 0.5–1.0 g of tissue was combusted into CO_2 and water with the aid of a Packard 306 Tri-Carb sample oxidizer. The ^3H was quantitatively recovered as $^3\text{H}_2\text{O}$ and counted with Monophase 40 as the liquid scintillator. On several occasions the ^3H radioactivity in the liver was determined either by direct combustion with the sample oxidizer or after digestion with Soluene-350 as described above for ^{32}P -containing samples. Both methods gave identical results. Soluene-350 and the scintillation fluids were all obtained from Packard Instruments Inc. All

radioactivity measurements were corrected for quenching by the use of an external standard.

Chemical analysis of serum HD lipoprotein

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma fraction V) as standard. Total cholesterol and phospholipids were measured as described by Röschlau *et al.* (1974) and by Bartlett (1959) respectively. For the determination of unesterified cholesterol the cholesteryl esterase was omitted from the assay medium. Cholesteryl esters were calculated as the difference between total cholesterol and unesterified cholesterol.

Results

Analysis of serum HD lipoprotein concentrations and chemical composition before and after partial hepatectomy

HD lipoprotein was isolated from serum of control animals and of partially hepatectomized rats and sham-operated controls 3 h after the operations. Protein, cholesterol, cholesteryl esters and phospholipids were measured in the isolated HD lipoprotein fractions. Table 1 shows that neither the concentration nor the composition of serum HD lipoprotein is affected significantly, if measured 3 h after partial hepatectomy. Neutral acylglycerols contribute only

Table 1. Serum HD lipoprotein concentration and composition in control rats, in partially hepatectomized rats and in sham-operated controls

The number of different sera is given in parentheses. All values are means $\pm$ S.E.M. The serum was isolated 3 h after partial hepatectomy or sham operation.

	Control rats (10)		Hepatectomized rats (3)		Sham-operated rats (3)	
	(mg/100 ml)	(wt. %)	(mg/100 ml)	(wt. %)	(mg/100 ml)	(wt. %)
Protein	68.2 $\pm$ 2.4	40.7 $\pm$ 0.5	61.2 $\pm$ 4.7	40.8 $\pm$ 0.4	65.7 $\pm$ 6.5	40.4 $\pm$ 1.0
Cholesterol	13.0 $\pm$ 0.7	7.8 $\pm$ 0.2	9.7 $\pm$ 1.0	6.5 $\pm$ 0.9	9.5 $\pm$ 1.4	5.9 $\pm$ 0.6
Cholesteryl esters	22.1 $\pm$ 1.6	13.0 $\pm$ 0.7	24.3 $\pm$ 1.2	16.4 $\pm$ 0.9	24.4 $\pm$ 2.5	15.0 $\pm$ 0.4
Phospholipids	64.9 $\pm$ 2.7	38.5 $\pm$ 0.7	55.1 $\pm$ 7.1	36.4 $\pm$ 2.0	62.3 $\pm$ 2.5	38.7 $\pm$ 1.7

Table 2. Distribution of radioactivity present in cholesteryl esters over serum lipoprotein fractions isolated after incubation of [^3H]cholesteryl ester-labelled HD lipoprotein with serum *in vitro* or after injection *in vivo*

All values are given as a percentage of total serum [^3H]cholesteryl esters (means $\pm$ S.E.M. for four analyses); every separate sample was subjected to lipid extraction followed by t.l.c. to separate the small amounts of unesterified [^3H]cholesterol from the bulk of [^3H]cholesteryl esters.

Distribution of [^3H]cholesteryl esters among serum lipoprotein fractions (%)

Lipoprotein fraction	Incubation <i>in vitro</i> for:			<i>In vivo</i> for 30 min in:	
	0 min	30 min	6 h	Sham-operated rats	Functionally eviscerated rats
VLD lipoprotein	1.1 $\pm$ 0.2	1.1 $\pm$ 0.2	1.3 $\pm$ 0.2	1.2 $\pm$ 0.3	1.2 $\pm$ 0.2
ID lipoprotein	0.9 $\pm$ 0.2	0.6 $\pm$ 0.3	0.7 $\pm$ 0.2	0.5 $\pm$ 0.2	0.6 $\pm$ 0.3
LD lipoprotein	5.8 $\pm$ 2.3	5.5 $\pm$ 1.3	3.6 $\pm$ 0.4	6.4 $\pm$ 2.4	5.7 $\pm$ 3.0
HD lipoprotein	92.2 $\pm$ 3.1	92.8 $\pm$ 2.6	94.4 $\pm$ 2.4	92.0 $\pm$ 4.4	92.4 $\pm$ 2.6

very little to rat serum HD lipoprotein (less than 1% by weight) and were not included in the Table. HD lipoprotein still has a normal composition if measured 8 h after partial hepatectomy, whereas at that time the concentration of serum HD lipoprotein is decreased by 13% (Van Tol *et al.*, 1978a).

Transfer of [^3H]cholesteryl esters from HD lipoprotein to other lipoprotein classes in vitro and in vivo

The screened [^3H]cholesteryl ester-labelled HD lipoproteins were incubated with serum at 37°C up to 6 h, followed by separation of the incubation medium by density-gradient ultracentrifugation as described before (Van Tol *et al.*, 1978a). Table 2 shows the distribution of radioactivity present in cholesteryl esters over the different serum lipoprotein fractions. The cholesteryl ester mass in VLD, ID and LD lipoprotein did not change during the 6 h incubation, whereas the cholesteryl esters of HD lipoprotein increased by $21 \pm 3\%$ (mean $\pm$ S.E.M. for four experiments). This increase was probably due to the action of phosphatidylcholine:cholesterol acyltransferase and coincided with a decrease in unesterified cholesterol.

When the labelled HD lipoprotein was injected *in vivo* into functionally eviscerated rats or sham-operated control animals, the distribution of [^3H]cholesteryl ester over the different serum lipoprotein fractions was identical when analysed 30 min after injection (see Table 2). No differences were detected between the distribution after incubation *in vitro* and circulation *in vivo*. Thus we found no evidence for exchange or transfer of [^3H]cholesteryl esters from biologically labelled HD lipoprotein to other serum lipoproteins, not even *in vivo* in the presence of accumulating triacylglycerol-rich lipoprotein remnants in the functionally eviscerated animals (Windler *et al.*, 1980).

Serum decay of ^3H -labelled HD lipoprotein cholesteryl esters

The screened HD lipoprotein was injected intravenously into partially hepatectomized or sham-operated rats and blood samples were taken during the next 6 h. At the end of the experiment the serum was analysed by density-gradient ultracentrifugation. At this time (6 h after injection) serum radioactivity was present for $76.0 \pm 2.2\%$ ($83.5 \pm 0.9\%$) in HD lipoprotein and for $15.6 \pm 1.3\%$ ($11.1 \pm 1.2\%$) in the LD lipoprotein (means $\pm$ S.E.M. for four experiments). The values in parentheses are for partially hepatectomized rats. In all density fractions more than 90% of the ^3H radioactivity was associated with cholesteryl esters.

In Fig. 1 the serum ^3H radioactivity is plotted semi-logarithmically against time. A biexponential serum decay is observed, with a short rapid phase

Fig. 1. Decay of serum radioactivity after intravenous injection of [^3H]cholesteryl ester-labelled HD lipoprotein into partially hepatectomized rats (O) or sham-operated controls (●).

The values are given as a percentage of the injected dose (means $\pm$ S.E.M. for four experiments). The t_1 of the slow phase is indicated.

Table 3. Kinetic parameters of [^3H]cholesteryl ester-labelled HD lipoprotein decay from serum of partially hepatectomized rats or sham-operated controls

The decay curve of each individual rat was analysed by the method of curve peeling and the kinetic parameters were calculated (see the Materials and methods section and Fig. 1). Values are the means $\pm$ S.E.M. for four experiments. Significant difference from the value in sham-operated rats is indicated by * ($P < 0.01$) or by ** ($P < 0.005$).

	Hepatectomized	Sham-operated
C_1	$0.99 \pm 0.03^*$	0.82 ± 0.06
t_1 (h)	$5.7 \pm 0.8^*$	4.2 ± 0.4
C_2	$0.02 \pm 0.03^*$	0.18 ± 0.06
t_2 (h)	0.1 ± 0.1	0.2 ± 0.1
F.T.R. (day^{-1})	$3.1 \pm 0.4^{**}$	4.8 ± 0.2
Intravascular (%)	$99 \pm 2^*$	83 ± 5

and a quantitatively more important slow phase. Removal of two-thirds of the liver resulted in a decrease in the compartment connected with the rapid phase (C_2) and significantly increased the t_1 of the slow phase. The decay curve of every separate experiment was analysed using a two-compartment model and some kinetic parameters are given in Table 3. C_1 and C_2 were determined graphically by extrapolation of the slow phase (Fig. 1) to zero time. After subtraction of the extrapolated slow phase

Table 4. Tissue radioactivities as measured 6 h after intravenous injection of [^3H]cholesteryl ester-labelled HD lipoprotein

All values are given as a percentage of the injected dose. Results are means $\pm$ S.E.M. for four analyses; each analysis was of tissue from a single rat. Significant difference from the value in sham-operated rats is indicated by * ($P < 0.01$) or by ** ($P < 0.001$).

	Tissue radioactivity (% of injected dose)			
	Hepatectomized		Sham-operated	
	(per total organ)	(per g of tissue)	(per total organ)	(per g of tissue)
Heart	0.3 ± 0.1	0.53 ± 0.09	0.3 ± 0.0	0.42 ± 0.04
Lungs	2.1 ± 0.4	1.71 ± 0.36	1.9 ± 0.3	1.50 ± 0.24
Spleen	1.6 ± 0.1	4.41 ± 0.35	1.5 ± 0.1	3.33 ± 0.21
Liver	$13.5 \pm 1.6^*$	$5.15 \pm 0.31^*$	34.0 ± 1.9	3.65 ± 0.20
Kidneys	1.2 ± 0.2	0.60 ± 0.09	1.2 ± 0.2	0.59 ± 0.11
Small intestine	12.3 ± 0.3	1.64 ± 0.05	15.2 ± 1.5	2.01 ± 0.19
Adipose tissue	8.1 ± 1.6	0.32 ± 0.06	6.0 ± 1.0	0.24 ± 0.04
Skeletal muscle	16.7 ± 3.4	0.15 ± 0.03	13.4 ± 2.1	0.12 ± 0.02
Skin	14.7 ± 0.1	0.32 ± 0.01	10.8 ± 1.5	0.24 ± 0.03
Serum	$44.3 \pm 0.9^*$	$4.19 \pm 0.07^{**}$	30.8 ± 0.7	2.89 ± 0.05

from the original decay curve, the rapid phase is obtained and the $t_{\frac{1}{2}}$ of both phases can be determined. The fraction of intravascular (radioactive) cholesteryl esters that is removed from the serum per day (F.T.R.) and the percentage of the radioactivity present in the intravascular compartment during the experiment were calculated by using the formulae given by Langer *et al.* (1972). Partial hepatectomy results in a 36% increase in the $t_{\frac{1}{2}}$ of the slow phase and a 38% decrease in the F.T.R.

Tissue radioactivities 6 h after the injection of ^3H -labelled HD lipoprotein cholesteryl esters

Table 4 shows the tissue radioactivities of nine major organs as measured 6 h after intravenous injection of [^3H]cholesteryl ester-labelled HD lipoprotein, expressed as a percentage of the injected dose per total organ and per g of tissue. The radioactivity lost from the serum compartment was quantitatively recovered in the tissues. It can be seen that most of the HD lipoprotein cholesteryl ester radioactivity removed from the serum of sham-operated rats is recovered in the liver. In this series of experiments the liver also contained the highest level of radioactivity if expressed per g of tissue. In similar experiments it was found that the adrenals accumulated also radioactivity from HD lipoprotein cholesteryl esters. If measured 4 h after injection their radioactivity level was 5% of the injected dose per g wet wt. (results not shown). Another tissue with a relatively high level of radioactivity is the small intestine, both per g of tissue and per total organ. Liver and small intestine together account for about 60% of the radioactivity removed from the circulation during the 6 h experiment. Partial hepatectomy results in a decrease in the total hepatic

radioactivity, as expected. Extrahepatic tissues show a tendency to contain a higher percentage of the injected dose after partial hepatectomy. The liver also contains more radioactivity after partial hepatectomy, if expressed per g of tissue. An exception to this general observation is the small intestine.

Serum decay of ^{32}P -labelled HD lipoprotein phospholipids

After intravenous injection of the ^{32}P -labelled HD lipoprotein phospholipids in sham-operated controls and partially hepatectomized rats multiple blood samples were taken over a period of 1 h and the serum radioactivities are plotted semi-logarithmically in Fig. 2. The ^{32}P -labelled HD lipoprotein phospholipids showed a monoexponential decay from serum with $t_{\frac{1}{2}}$ of 0.71 ± 0.03 h for the sham-operated controls and a $t_{\frac{1}{2}}$ of 1.5 ± 0.1 h for the partially hepatectomized rats. At the end of the experiment the serum was analysed by density-gradient ultracentrifugation and it was observed that 66.2 ± 0.7 and $70.7 \pm 1.3\%$ of the ^{32}P label was associated with HD lipoprotein for sham-operated controls and partially hepatectomized rats respectively. Some radioactivity was recovered in LD lipoprotein (5–8%) and at $d > 1.21$ g/ml (20–25%), but very little radioactivity was present at $d < 1.02$ g/ml. Of the ^{32}P radioactivity in HD lipoprotein 80–86% was associated with phosphatidylcholine and 12–18% with sphingomyelin at the end of the 1 h experiment. The F.T.R. per h was determined from the decay of individual rats, as described above, and the kinetic parameters are given in Table 5. Partial hepatectomy induces a 108% increase in the $t_{\frac{1}{2}}$ and a 54% decrease in the F.T.R. of labelled phospholipids.

Fig. 2. Decay of serum radioactivity after intravenous injection of ^{32}P -phospholipid-labelled HD lipoprotein into partially hepatectomized rats (○) or sham-operated controls (●).

The values are given as a percentage of the injected dose (means $\pm$ S.E.M. for three experiments). The $t_{1/2}$ is as indicated.

Table 5. Kinetic parameters of ^{32}P -phospholipid-labelled HD lipoprotein decay from serum of partially hepatectomized rats or sham-operated controls

Values are the means $\pm$ S.E.M. for three experiments. For further details see the Materials and methods section and Fig. 2. Significant difference ($P < 0.001$) from the value in sham-operated rats is indicated by *.

	Hepatectomized	Sham-operated
$t_{1/2}$ (h)	$1.48 \pm 0.11^*$	0.71 ± 0.03
F.T.R. (h^{-1})	$0.49 \pm 0.04^*$	1.06 ± 0.05

Tissue radioactivities 1 h after injection of ^{32}P -labelled HD lipoprotein phospholipids

At the end of the experiments three organs (liver, adipose tissue and skeletal muscle) were obtained as described above and analysed for ^{32}P radioactivity after tissue destruction with Soluene-350 (see the Materials and methods section). Blood was collected in EDTA and the erythrocytes were washed once in saline before destruction. The ^{32}P tissue radioactivities, expressed as a percentage of the injected dose per total organ and per g of tissue, are shown in Table 6.

The recovery of the injected ^{32}P radioactivity in serum and the tissues analysed was $91 \pm 5\%$ for the sham-operated controls and $93 \pm 4\%$ for the partially hepatectomized rats (means $\pm$ S.E.M. for three experiments). It is clear that in sham-operated controls almost 75% of the phospholipid radioactivity removed from serum is recovered in the liver.

Discussion

From the work of Goldstein & Brown (1974) on the human fibroblast it is concluded that LD lipoprotein catabolism involves binding to a receptor, internalization of the particle and degradation of the individual components. Miller *et al.* (1977) proposed a similar sequence for HD lipoprotein. One way of testing this hypothesis *in vivo* is to compare the F.T.R. rates of the different HD lipoprotein components. These rates have to be equal if the lipoprotein particle is catabolized only as one single unit. Roheim *et al.* (1972) found equal rates of removal of the different HD apolipoproteins from plasma and Sigurdsson *et al.* (1979) showed that the uptake of the cholesteryl ester and apolipoprotein moieties of HD lipoprotein by the perfused liver were both low, but comparable. However, Eisenberg *et al.* (1973) found some differences between the catabolic rates of the different HD apolipoproteins in the rat. Drevon *et al.* (1977) reported a substan-

Table 6. Tissue radioactivities as measured 1 h after intravenous injection of ^{32}P -labelled HD lipoprotein

All values are expressed as a percentage of the injected dose. Results are means $\pm$ S.E.M. for three analyses; each analysis was of tissue from a single rat. Significant difference ($P < 0.01$) from the value in sham-operated rats is indicated by *.

	Tissue radioactivity (% of injected dose)			
	Hepatectomized		Sham-operated	
	(per total organ)	(per g of tissue)	(per total organ)	(per g of tissue)
Liver	$16.7 \pm 3.8^*$	5.97 ± 0.70	46.0 ± 1.7	5.24 ± 0.20
Adipose tissue	2.0 ± 0.0	0.09 ± 0.01	1.7 ± 0.3	0.05 ± 0.01
Skeletal muscle	6.3 ± 0.3	0.05 ± 0.01	3.7 ± 1.2	0.03 ± 0.01
Erythrocytes	2.7 ± 0.3	0.26 ± 0.04	3.0 ± 0.0	0.25 ± 0.01
Serum	$65.3 \pm 1.9^*$	$6.09 \pm 0.10^*$	37.0 ± 2.1	3.47 ± 0.22

tial uptake of HD lipoprotein cholesteryl esters by isolated liver cells. From their experiments *in vitro* they calculated a possible half-life *in vivo* of about 30 min for the cholesteryl ester component of HD lipoprotein, compared with a half-life of 6–10 h for HD apolipoprotein (Roheim *et al.*, 1971; Eisenberg *et al.*, 1973; Van Tol *et al.*, 1978a).

In a previous study we found that the degradation rate *in vivo* of iodinated HD apolipoproteins A and C was not influenced by partial hepatectomy (Van Tol *et al.*, 1978a), whereas this operation resulted in a marked decrease in the F.T.R. of iodinated LD apolipoprotein (Van Tol *et al.*, 1978b). This suggests that the major HD apolipoproteins are hardly catabolized by the liver *in vivo*, a conclusion supported by the findings of Sigurdsson *et al.*, (1979) and Carew *et al.* (1976).

The data in the present paper show a significant effect of partial hepatectomy on the F.T.R. of HD lipoprotein cholesteryl esters. The F.T.R. of radioactive cholesteryl esters is significantly higher than our previously reported values for HD apolipoprotein. Only the turnover of the cholesteryl esters is decreased after partial hepatectomy (see Table 7). These findings show that the apoprotein and cholesteryl ester moieties of HD lipoprotein may have partly separate metabolic pathways.

By screening we obtained HD lipoprotein labelled almost exclusively in the cholesteryl ester moiety. It is generally accepted, at least in the rat, that the cholesteryl esters are one of the least exchangeable lipid classes (Roheim *et al.*, 1963; Goodman, 1964; Barter & Lally, 1978). As mentioned before we also found no evidence for exchange or transfer of [³H]cholesteryl esters from HD lipoprotein to other lipoproteins in rat serum, using models either *in vivo* or *in vitro* (see Table 2).

The inhibiting effect of partial hepatectomy on the F.T.R. of HD lipoprotein cholesteryl esters can therefore be explained by a difference between the cellular degradation characteristics of the cholesteryl ester moiety and the protein moiety of the same HD lipoprotein particle. During the 1 h screening procedure we eliminated [³H]cholesteryl esters that are associated with the apolipoprotein E-containing HD lipoprotein, a postulated subfraction of HD lipoprotein. This subfraction has a very rapid turnover *in vivo* with a half-life of only a few minutes (Mahley *et al.*, 1979). The lipid and protein moieties of this minor HD lipoprotein subfraction appear to be taken up in parallel by the perfused liver (Quarfordt *et al.*, 1980). Of course a hypothetical transfer of HD lipoprotein cholesteryl ester from the bulk of HD lipoprotein (containing apolipoprotein A as its major protein component) to the apolipoprotein E-containing subfraction could provide an alternative explanation for the discrepancies between the F.T.R. of the protein and cholesteryl ester moieties of HD lipoprotein observed by us. As rat serum is virtually devoid of cholesteryl ester transfer and exchange activities (Barter & Lally, 1978; Table 2) this is highly unlikely.

Re-secretion by the liver of cleared radioactive cholesteryl esters and phospholipids into newly synthesized VLD lipoprotein occurred only to a small extent. This was demonstrated by the very low level of ³H or ³²P radioactivity in the VLD lipoprotein density range at the end of the experiments. The specific radioactivities of total liver cholesterol and phospholipid were 15% and 5% of the values for serum HD lipoprotein respectively.

The results shown in Table 6 point to a very active hepatic uptake of radioactive HD lipoprotein phospholipids. Phospholipids, and phosphatidylcholine in particular, are known to exchange between cell membranes and serum lipoproteins. This phenomenon may explain (part of) the high hepatic radioactivity and the effect of partial hepatectomy on the F.T.R. of radioactive phospholipids, as this operation results in a 66% decrease in the exchangeable hepatic phospholipid pool. Metabolism of radioactive HD lipoprotein phospholipids by the serum enzyme phosphatidylcholine:cholesterol acyltransferase and subsequent hepatic uptake of ³²P-labelled lysophosphatidylcholine can also explain part of the observed phospholipid turnover and hepatic radioactivity (cf. Stein & Stein, 1966; Lacko *et al.*, 1974).

HD lipoprotein phospholipids can also be hydrolysed by heparin-releasable liver lipase (Van Tol *et al.*, 1980). This enzyme is bound at the surface of non-parenchymal (endothelial) liver cells, both *in vivo* and *in vitro* (Jansen *et al.*, 1978; Kuusi *et al.*, 1979). The non-parenchymal liver cells, in addition to the hepatocytes, are also active in the

Table 7. Distinction between the turnover rates of the major apolipoproteins (A and C) and the cholesteryl ester moiety of serum HD lipoprotein in rats

Results given are F.T.R. per day (means $\pm$ S.E.M. for four experiments). The values for HD apolipoprotein turnover are taken from Van Tol *et al.* (1978a) and the values for HD lipoprotein cholesteryl ester turnover are from Table 3. Significant difference ($P < 0.01$) from the value in sham-operated rats is indicated by *. Significant difference ($P < 0.001$) from the value for HD apolipoprotein is indicated by **.

	F.T.R. (day ⁻¹)	
	Hepatectomized	Sham-operated
HD apolipoprotein A and C	2.8 $\pm$ 0.1	2.9 $\pm$ 0.2
HD lipoprotein cholesteryl ester	3.0 $\pm$ 0.4*	4.8 $\pm$ 0.2**

Fig. 3. Hypothetical mechanism for the hepatic uptake of serum HD lipoprotein cholesteryl (esters)

This model includes a 'fusogenic' HD lipoprotein particle (containing increased concentrations of lysophosphatidylcholine and non-esterified fatty acids) that is generated by the phospholipase action of heparin-releasable liver lipase. During a reversible fusion with the liver cell membrane, part of the core cholesteryl esters and surface (lyso)phosphatidylcholine and unesterified cholesterol are taken up by the liver cell and HD lipoprotein₂ is converted into HD lipoprotein₃. Abbreviations used: A and C, apolipoprotein A and C; Ch(E), cholesteryl (esters); PL, phospholipid (predominantly phosphatidylcholine); FFA, non-esterified fatty acids; Ch, cholesterol.

uptake of HD lipoprotein cholesteryl esters from the circulation (Van Berkel & Van Tol, 1979). Inhibition of heparin-releasable liver lipase *in vivo* results in a substantial rise in the concentration of HD lipoprotein₂ (especially the phospholipids) and a decrease in HD lipoprotein₃ lipids (Jansen *et al.*, 1980). On the basis of these observations they proposed a physiological role for this enzyme in the conversion of HD lipoprotein₂ into HD lipoprotein₃. The proposed mechanism included, in addition to the phospholipase action, a hypothetical transfer of cholesteryl (esters) to other lipoproteins or tissues (Van Tol *et al.*, 1980). The hepatic uptake of HD lipoprotein cholesteryl esters, described in the present paper, may be incorporated into the conversion mentioned above. This results in the mechanism shown in Fig. 3. If the phospholipase activity of heparin-releasable liver lipase, which can be measured *in vitro* with HD lipoprotein phosphatidylcholine as substrate (Van Tol *et al.*, 1980), is equal to the activity *in vivo*, it can be calculated that more than 50% of the very high F.T.R. of radioactive HD lipoprotein phospholipids in normal rats (1.06/h, Table 5) is explained by the action of heparin-releasable liver lipase. The rapid hepatic uptake of radioactive HD lipoprotein phospholipids may thus involve hydrolysis of phosphatidylcholine

to lysophosphatidylcholine and subsequently uptake of lysophosphatidylcholine by the liver (cf. Stein & Stein, 1966). As illustrated in Fig. 3, the lysophosphatidylcholine could play a role in the uptake mechanism of the HD lipoprotein cholesteryl esters present in the core of the particle by inducing a reversible fusion between HD lipoprotein and the plasma membrane of liver cells. Such a mechanism could account for the cellular uptake of the core cholesteryl esters without the concomitant uptake of substantial amounts of HD apolipoproteins.

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