The uptake and metabolism of chylomicron-remnant lipids by rat liver parenchymal and non-parenchymal cells *in vitro*

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The uptake and metabolism of chylomicron-remnant lipids by individual liver cell types was examined by incubating remnants with monolayer cultures of hepatocytes, Kupffer cells, and endothelial cells from rat liver. Remnants were prepared *in vitro* from radiolabelled mesenteric-lymph chylomicra, utilizing either purified lipoprotein lipase from bovine milk, or plasma isolated from heparinized rats. The resulting particles contained [^3H]phosphatidylcholine and cholesterol, and [^{14}C]oleate in the acylglycerol, phospholipid, fatty-acid and cholesterol-ester fractions. The capacities of the three cell types for uptake of both [^3H]lipids and [^{14}C]lipids were determined to be, on a per-cell basis, in the order: Kupffer > hepatocytes > endothelial. The relative proportions of [^3H]phospholipid and total [^3H]cholesterol taken up by hepatocytes and non-parenchymal cells remained constant with time. The uptake of [^{14}C]oleoyl lipids by all three cell types was slightly greater than that of the total [^3H]cholesterol and [^3H]phospholipid components. There was evidence of cholesterol-ester hydrolysis and turnover of [^{14}C]oleate in the phospholipid fraction in hepatocytes and Kupffer cells, but not endothelial cells, over the first 2 h. With both remnant preparations, these observations indicate that significant differences exist between the three major liver cell types with respect to the uptake and metabolism of remnant lipid components.

INTRODUCTION

The liver plays a central role in the uptake and metabolism of various serum lipoprotein components (Langer *et al.*, 1970; Hay *et al.*, 1971; Smith *et al.*, 1978; Nicoll *et al.*, 1980; Van Tol & Van Berkel, 1980), including remnant particles derived from extrahepatic catabolism of triacylglycerol-rich lipoproteins (Redgrave, 1970; Faergeman & Havel, 1975). However, the liver consists primarily of parenchymal (hepatocytes) Kupffer and endothelial cells. The hepatocytes account for roughly 90% of the liver mass, but only about two-thirds of the total cell population (Van Berkel, 1979). Although the roles of the individual cell types in lipoprotein catabolism have not been completely defined, studies have shown that both parenchymal and non-parenchymal cells can take up and metabolize lipoprotein constituents (Van Tol & Van Berkel, 1980; Van Berkel & Van Tol, 1978, 1979). It has been proposed that non-parenchymal cells can degrade lipoproteins, since they are enriched in cathepsin D and acid lipase (Van Berkel *et al.*, 1975). Evidence exists which suggests that non-parenchymal cells can metabolize apoproteins and cholesterol ester in high- and low-density lipoproteins (Van Berkel & Van Tol, 1978, 1979). Similarly, endothelial cells were implicated in the uptake and degradation of acetyl-low-density lipoproteins, as part of a scavenger pathway (Nagelkerke *et al.*, 1983). In previous studies, utilizing perfused liver *in situ*, we observed that both parenchymal and non-parenchymal cells take up and metabolize chylomicron-remnant lipid constituents (Lippiello *et al.*, 1981). The results suggested that differences exist in the metabolism of individual

remnant lipids by the different cell types. However, the methods used did not allow for simultaneous comparison of all three cell types.

The present study directly compares the uptake and metabolism of remnant lipids by the three major liver cell types by using monolayer cultures of hepatocytes, Kupffer cells and endothelial cells. The results confirm that both parenchymal and non-parenchymal cells take up and metabolize chylomicron-remnant lipids and that significant differences exist between the cell types in handling individual remnant lipid components, regardless of the type of remnant lipoprotein preparation used.

EXPERIMENTAL PROCEDURES

Chylomicron and remnant preparation

[*N*-methyl- ^3H]Phosphatidylcholine, [*carboxy*- ^{14}C]trioleoylglycerol, and [$1\alpha,2\alpha(n)$ - ^3H]cholesterol were introduced into 250–300 g Sprague–Dawley rats by duodenal catheter, as previously described (Lippiello *et al.*, 1981). Radiolabelled chylomicra were isolated from the mesenteric lymph and purified by agarose-gel chromatography as described by Imaizumi *et al.* (1978). Remnant lipoproteins were produced *in vitro* by two methods: incubation of radiolabelled chylomicra with LPL purified from milk (LPL remnants; El-Maghrabi *et al.*, 1978) or incubation of chylomicra with plasma from post-heparinized rats (PHP remnants; Eisenberg & Rachmilewitz, 1975). Since some variability existed in composition between remnant preparations, all experiments presented below represent a single remnant

Abbreviations used: LPL, lipoprotein lipase; PHP, post-heparin plasma; SDS, sodium dodecyl sulphate; GBS, Gey's balanced salts; DMEM, Dulbecco's modified Eagle's medium.

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Table 1. Distribution of radiolabelled lipids in chylomicra and remnants

Total lipids were extracted from chylomicra and remnants and separated into individual classes by t.l.c. as described in the Experimental procedures section. The distribution of each label was calculated as a percentage of the total recovered from chromatographic plates.

Lipid fraction	Chylomicra		LPL remnants		PHP remnants	
	³ H	¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C
Phospholipid	23	1	13	< 1	9	< 1
Monoacylglycerol	—	1	—	3	—	3
Diacylglycerol	—	6	—	8	—	8
Non-esterified cholesterol	29	—	24	—	27	—
Fatty acid	—	5	—	13	—	16
Triacylglycerol	—	87	—	75	—	72
Cholesterol ester	45	—	63	—	63	—

preparation of each type, utilized in duplicate experiments. Control experiments without cells but in the presence of albumin showed no LPL remaining with the remnants. Likewise, SDS/polyacrylamide-gel electrophoresis did not show the presence of any proteins from the LPL preparations.

Isolation and maintenance of liver cells

Hepatocytes were isolated from the livers of 250–300 g male Sprague-Dawley rats (Berry & Friend, 1969; Seglen, 1972) with minor modifications. The liver was perfused first with Ca²⁺-free GBS for 5 min, and then with GBS containing Ca²⁺ and 0.05% collagenase for 10–20 min at a flow rate of 30–40 ml/min. All buffers were saturated with O₂/CO₂ (19:1). The liver was removed, gently minced, and incubated for 15 min at 37 °C in the collagenase solution. The resulting suspension was filtered through several layers of cheesecloth or nylon mesh, and sedimented by centrifugation at 50 g for 2 min. The cell pellet was washed three times by resuspension in GBS and sedimentation at 50 g. Parenchymal cells prepared by this method were determined to be 90–95% pure (Seglen, 1976). Cell viability was typically 75–90%, determined by exclusion of 0.25% Trypan Blue. Cells were seeded on 35 mm-diameter Falcon plastic Petri dishes at a density of 1 × 10⁶ viable cells/plate. Before use, cells were maintained for 1–2 days in DMEM with L-glutamine, containing 5% (v/v) foetal-calf serum, supplemented with 1% insulin and penicillin/streptomycin.

Kupffer and endothelial cells were isolated by the methods of Knook *et al.* (1977). Livers were first perfused with GBS for 5 min and then with GBS containing 0.2% Pronase for 10–15 min at a flow rate of 30 ml/min. The liver was excised, minced, and incubated in the Pronase solution for 1 h at 37 °C to destroy parenchymal cells. Total non-parenchymal cells were sedimented by centrifugation at 300–350 g for 10 min, resuspended in GBS, and isolated on a 17.5% metrizamide gradient. Purified Kupffer- and endothelial-cell fractions were obtained by centrifugal elutriation (Knook *et al.*, 1977). Cell viability was greater than 90%, determined by exclusion of 0.25% Trypan Blue. Cell purity was determined cytochemically by peroxidase staining with 0.1% diaminobenzidine, which is specific for Kupffer cells (Knook *et al.*, 1977). Cross-contamination was typically 10–15%, by the difference of non-staining cells from the total. The yield

of non-parenchymal cells from a single liver was 20–30%. Kupffer cells were seeded at a density of 1 × 10⁶ viable cells/plate, and before use were maintained 1–2 days in DMEM with L-glutamine, containing 20% foetal-calf serum, 20 mM-Hepes, pH 7.4, penicillin/streptomycin and 1% insulin. Endothelial cells were seeded at a density of 1 × 10⁶ viable cells and maintained for 1–2 days in DMEM containing 30% (v/v) rat serum as described by de Leeuw *et al.* (1982).

Incubation procedures

PHP remnants or LPL remnants (both 20 µg of phospholipid) were suspended in 1 ml of DMEM, added to culture plates, and incubated at 37 °C in a humidified atmosphere of air/CO₂ (19:1) for various periods of time. Incubations were terminated by aspiration of the fluids, followed by three washes with ice-cold GBS. Cells were then scraped from the plates and suspended in GBS. Portions were taken for protein determination by the method of Lowry *et al.* (1951) and extraction of cellular lipids for t.l.c. and radiolabel analyses. Results presented below are normalized to 1 × 10⁶ cells on the basis of the estimated protein content of parenchymal and non-parenchymal cells (Knook & Sleyster, 1980). The results presented are from a single set of experiments which are representative of three separate studies. All incubations were in duplicate. The variation between duplicates was less than 15%.

Lipid analyses

Cellular lipids were extracted by the method of Bligh & Dyer (1959) and were separated by t.l.c. on silica-gel H, a hexane/diethyl ether/formic acid (20:10:1, by vol.) solvent system. Phospholipid (Chalvardjian, 1970), total acylglycerols (Sardesai & Manning, 1968) and cholesterol (Lippielo *et al.*, 1979) mass analyses were performed as described by the authors cited.

Electrophoresis

Protein extracts from chylomicra, PHP remnants and LPL remnants and standards were run on polyacrylamide slab gels (4–30%, w/v) (King & Laemmli, 1971). Approx. 50 µg of protein were applied in all cases.

Materials

Cell-culture materials were obtained from Flow Laboratories, McLean, VA, U.S.A. Bovine serum albumin (fatty-acid-free) was purchased from Miles

Laboratories, Elkhart, IN, U.S.A. Metrizamide, Pronase ('protease XIV bacterial') and collagenase (type IA) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. [*Carboxy*- ^{14}C]Trioleoylglycerol and [$1\alpha,2\alpha(n)$ - ^3H]cholesterol were obtained from Amersham Corp., Arlington Heights, IL, U.S.A. [*N-methyl*- ^3H]Phosphatidylcholine was synthesized from egg lecithin and [^3H]methyl iodide (Stoffel, 1975). Egg lecithin (type V-E) was purchased from Sigma, and [^3H]methyl iodide was obtained from New England Nuclear. All other chemicals were of analytical grade and purchased commercially.

RESULTS

Characterization of chylomicra and remnants

The composition of isolated chylomicra, as a percentage of total mass, was 93% triacylglycerol, 5% phospholipid, 1% total cholesterol and 1% protein. All of the major lipid classes were labelled (*in vivo*) as follows: [^{14}C]oleoyl mono-, di- and tri-acylglycerol, phospholipid, cholesterol ester and fatty acid, [*N-methyl*- ^3H]phospholipid, [$1\alpha,2\alpha(n)$ - ^3H]cholesterol and cholesterol ester. The relative distribution of radiolabelled lipids in these chylomicra was determined by t.l.c. and is presented in Table 1.

In the present experiments, remnant lipoproteins produced by incubating radiolabelled chylomicra with purified lipoprotein lipase (LPL remnants) contained, as a percentage of total mass, 76% total acylglycerols, 7% phospholipid, 3% total cholesterol and 14% protein. Remnants produced by incubating chylomicra with plasma from heparinized rats (PHP remnants) contained 83% total acylglycerols, 4% phospholipid, 2% total cholesterol and 11% protein. Changes in lipid composition were reflected in the distribution of radiolabels among the major lipid classes (Table 1) and were similar to those reported by Nilsson *et al.* (1981) for remnants produced *in vitro* in the presence or absence of serum.

Remnants were seen to be depleted of triacylglycerol and enriched in total cholesterol when the mass composition was compared with that of the chylomicra. The distribution of radiolabelled lipids shows that the cholesterol-ester content of remnants was increased compared with chylomicra (Table 1). From changes in composition and radiolabel distributions it can be estimated that the percentages of triacylglycerol hydrolysis for PHP and LPL remnants were 50% and 90% respectively. From surface-to-volume considerations it can be calculated that there is a decrease in diameter, relative to chylomicra, of approx. 20% for PHP remnants and 50% for LPL remnants.

Polyacrylamide-gel electrophoresis of apoprotein revealed differences between both types of remnant and chylomicra (Fig. 1). The amount of protein applied to the gel was limited so that the changes in the apoprotein E region would be easily detected. Specifically, both PHP and LPL remnants contained relatively less apolipoprotein A-I. Both contained some C apolipoproteins as well, although the apoprotein B at the top of the gel normally found in remnants (El-Maghrabi *et al.*, 1978) is not easily seen. LPL remnants contained albumin and some apolipoprotein E. The major difference appeared to be a significant enrichment of apolipoprotein E in PHP remnants, in agreement with previous observations (Nilsson *et al.*, 1981).

Fig. 1. Electrophoresis of protein extracts from PHP and LPL remnants

Total protein extracts ($\sim 50 \mu\text{g}$) were subjected to electrophoresis on polyacrylamide slab gels as described in the Experimental procedures section. (a) Protein standards: A-I, apolipoprotein A-I; E, apolipoprotein E; C, apolipoprotein C; Alb, bovine serum albumin; (b) chylomicra; (c) remnants prepared with purified lipoprotein lipase; (d) remnants prepared with serum from heparinized rats.

Cellular-uptake studies

To monitor remnant-lipid uptake, cells were incubated for up to 2 h with equivalent amounts of PHP or LPL remnants ($20 \mu\text{g}$ of phospholipid). The results from a representative set of experiments are presented in Fig. 2. The uptake of both [^3H]lipids and [^{14}C]lipids was nearly linear for up to 2 h in all three cell types, with both PHP and LPL remnants. There was always some radiolabelled lipid associated with the cells at zero time (1–2%), owing to the time necessary for addition and withdrawal of substrate and washes with buffer. All uptake experiments were performed at saturating concentrations of remnant lipid [i.e., with twice the concentration of remnant phospholipid ($40 \mu\text{g}$)], the percentages of radiolabelled lipid taken up were approximately one-half those shown in Fig. 2]. Therefore the results of Fig. 2 represent the relative maximal uptake capacities for the three cell types, which are in the following order: Kupffer > hepatocytes > endothelial. The relative uptake of ^3H -labelled components by the three cell types in 2 h, based on equal numbers of cells, was roughly 1.7:1:0.3 (by vol.) for PHP remnants and 2.3:1:0.5 (by vol.) for LPL remnants.

The uptake of [^{14}C]oleoyl lipids by all cell types was slightly higher than that of the [^3H]lipid components, with both types of remnant particle. The uptake of [^{14}C]oleoyl lipids was greatest in hepatocytes, especially

Fig. 2. Uptake of total ^3H - and ^{14}C -labelled remnant lipids by parenchymal and non-parenchymal cells

Hepatocytes, Kupffer cells and endothelial cells were incubated with radiolabelled remnant lipoproteins in duplicate for various lengths of time as described in the Experimental procedures section. The results are expressed as a percentage of the total radioactivity (d.p.m.) present in the incubation mixture taken up by 10^6 cells. PHP, remnants prepared with post-heparin plasma; LPL, remnants prepared with purified lipoprotein lipase; ●, Kupffer cells; ○, hepatocytes; △, endothelial cells.

Table 2. Metabolism of individual remnant lipids by parenchymal and non-parenchymal cells

Isotopic ratios for individual lipid species were determined for the experiments presented in Fig. 2, after separation of the major lipid classes by t.l.c. (see the Experimental procedures section).

	$\frac{[^3\text{H}]\text{Esterified cholesterol}^*}{[^3\text{H}]\text{Non-esterified cholesterol}}$		$\frac{[^{14}\text{C}]\text{Phospholipid}^\dagger}{[^3\text{H}]\text{Phospholipid}}$	
	PHP remnants	LPL remnants	PHP remnants	LPL remnants
Remnants	2.3	2.6	0.4	0.2
Hepatocytes				
0 min	2.3	2.5	0.5	0.4
30 min	1.8	1.7	0.7	0.8
120 min	1.5	1.4	0.9	1.0
Kupffer				
0 min	2.0	2.0	0.5	0.5
30 min	1.9	1.9	0.7	0.8
120 min	1.6	1.7	1.1	1.4
Endothelial				
0 min	2.0	1.5	0.4	0.3
30 min	2.1	1.5	0.5	0.3
120 min	2.0	1.4	0.4	0.4

* The ratio of radiolabel associated with cholesterol and cholesterol-ester fractions after separation on chromatographic plates.

† The internal ratio for double-labelled phospholipid recovered from chromatographic plates; ^{14}C = ^{14}C oleate; ^3H = $[\text{carboxy-}^3\text{H}]\text{phospholipid}$.

Table 3. Uptake of ³H-labelled remnant lipids by parenchymal and non-parenchymal cells

Hepatocytes, Kupffer cells and endothelial cells were incubated with remnant lipoproteins for 20 min and the total cellular lipids were extracted and separated into the main lipid classes by t.l.c. as described in the Experimental procedures section. The results are presented as the percentage of the total radioactivity in [³H]lipid (d.p.m.) present in incubations that was associated with either the phospholipid or the combined cholesterol/cholesterol-ester fractions in each cell type/10⁶ cells. Values are the means of duplicates and were determined from the data of the experiments presented in Fig. 2.

Cells	Radioactivity (%)			
	PHP remnants*		LPL remnants†	
	Phospholipid	Total cholesterol	Phospholipid	Total cholesterol
Hepatocytes				
0 min‡	0.2	1.8	0.3	1.8
120 min	1.2	10.5	1.2	6.7
Kupffer				
0 min	0.2	1.8	0.3	1.9
120 min	2.0	16.9	2.6	16.1
Endothelial				
0 min	0.2	1.6	0.2	1.1
120 min	0.4	3.5	0.9	5.0

* Remnants produced by incubation of chylomicra with PHP.

† Remnants produced by incubation of chylomicra with purified lipoprotein lipase.

‡ Results for '0 min' represent material bound to cells after addition and immediate withdrawal of remnant material and several washes with ice-cold buffer, which typically required less than 1 min.

Table 4. Uptake of phospholipid, total cholesterol and total fatty acid by parenchymal and non-parenchymal cells

Hepatocytes, Kupffer cells, and endothelial cells were incubated with remnant lipoproteins for 20 min and the total cellular lipids were extracted and separated into the main lipid classes by t.l.c. as described in the Experimental procedures section. These data were calculated from the data in Fig. 2 ([¹⁴C]fatty acid and triacylglycerol) and Table 3 (phospholipid and total cholesterol).

Cells	Uptake (μg/10 ⁶ cells)*					
	PHP remnants			LPL remnants		
	Phospholipid	Cholesterol	Fatty acid†	Phospholipid	Cholesterol	Fatty acid
Hepatocytes	2.0	1.0	66	1.8	0.4	22
Kupffer	3.6	1.4	88	4.6	1.1	42
Endothelial	0.4	0.2	11	1.4	0.3	92

* Corrected for zero-time controls.

† Sum of triacylglycerol and non-esterified fatty acid (total ¹⁴C in Fig. 2).

with PHP remnants. The absolute amount of [¹⁴C]oleate taken up in excess of the [³H]lipids varied and depended in part on the quantity of fatty acid and acylglycerol present in the remnant preparation. Examination of the post-incubation fluids showed that all of the neutral-lipid fractions, including triacylglycerol and free fatty acid, were depleted to the same extent (results not shown), suggesting that triacylglycerol hydrolysis also contributed to [¹⁴C]oleate taken up by the cells. In general, the results obtained for the uptake of total remnant lipid were similar with PHP and LPL remnants, despite variations in apolipoprotein composition (see Fig. 1).

Control experiments were run to determine the

specificity of the association of lipoproteins with the different cell types. When compared with the PHP remnants the percentages of ¹⁴C radioactivity of the chylomicron that became associated with the cells were: hepatocytes, 18%; endothelial cells, 37%; Kupffer cells, 61%. Since the volume of the chylomicra is about 15 times greater than the PHP remnants used, the number of chylomicron particles bound would amount to about 4% of the number of PHP remnants. On the basis of these calculations it would appear that the binding of the remnants was rather specific.

In general, the results indicated metabolic turnover of all of the neutral-lipid fractions in both parenchymal and

non-parenchymal cells. There were no major differences between PHP and LPL remnants. Changes in the distribution of [^{14}C]oleate were similar to those seen in perfused liver (Lippielo *et al.*, 1981), with a slow redistribution of [^{14}C]oleate from the monoacylglycerol and non-esterified-fatty-acid fractions into diacylglycerol and triacylglycerol over the first 2 h (results not shown).

Variations in isotopic ratios indicated metabolic turnover of remnant phospholipid and cholesterol ester (Table 2). There was appreciable hydrolysis of cholesterol ester in hepatocytes and Kupffer cells over the first 2 h, as evidenced by the [^3H]esterified/[^3H]non-esterified cholesterol ratio, which decreased by approx. 40% in hepatocytes and 20% in Kupffer cells. This was seen with both PHP and LPL remnants. There appeared to be little, if any, change in this ratio in endothelial cells. Similarly, there was a 2–3-fold increase in the [^{14}C]oleoyl/[N -methyl- ^3H]phospholipid ratio in hepatocytes and Kupffer cells with both remnant preparations, but very little change in endothelial cells. Therefore, despite a reproducible and significant uptake of remnant particles by endothelial cells (Fig. 2), metabolism of phospholipid and cholesterol ester over the first 2 h was limited.

The [^3H]esterified/[^3H]non-esterified cholesterol ratio in Kupffer and endothelial cells was lower than that of either PHP or LPL remnants. This suggests that these cells may bind remnant particles that contain relatively less cholesterol ester. This was also observed in liver-perfusion studies, where non-parenchymal cells accumulated less cholesterol ester than did hepatocytes (Lippielo *et al.*, 1981). This is probably not due to adsorption of non-esterified cholesterol from the medium, since the total cholesterol/phospholipid ratio does not change appreciably from that of the remnant substrates in 2 h (Table 3). In fact the data of Table 3 suggest that the remnant core and surface components are both taken up at the same rate, since the cell-associated [^3H]phospholipid and total [^3H]cholesterol were increased proportionally. This was true for all three cell types, with both types of remnants.

The amount of each major lipid class taken up by the three cell types was estimated on the basis of the percentage of each labelled compound that became cell-associated and the mass of each lipid present in the particle (Table 4). Although the relative amounts of uptake seen are reflected in percentage uptake, two things should be noted. First, on a mass basis the bulk of the lipid taken up is the acyl component, derived from triacylglycerol and non-esterified fatty acid; second, much more of the acyl moiety was taken up from PHP remnants than from LPL remnants. This is probably because twice as much triacylglycerol was present in the PHP preparation (369 as against 184 μg per incubation). Such a result would be expected if the intact particle was endocytosed.

DISCUSSION

In general, the present findings are consistent with previous results suggesting that both parenchymal and non-parenchymal cells take up and metabolize chylomicron-remnant lipids (Lippielo *et al.*, 1981).

The relative capacities of the three cell types for the uptake of total remnant lipids are Kupffer > hepatocytes > endothelial, regardless of the remnant preparation used. On the basis of the [^3H]lipid components,

cholesterol and phospholipid, the mean ratio for uptake of PHP and LPL remnants per cell was: hepatocytes/Kupffer/endothelial, 1:2:0.4. It has been estimated that the relative numbers of each cell type in the intact liver are: hepatocytes/Kupffer/endothelial, 10:1:4 (by vol.) (Knook & Sleyster, 1980). Together with the present results this predicts that the relative contributions of the total cell populations to remnant uptake *in situ* should be roughly 10:2:1.6 (by vol.) for hepatocytes, Kupffer cells and endothelial cells respectively. In liver-perfusion studies (Lippielo *et al.*, 1981) the distribution was 10:2.4:1 (by vol.), in good agreement with the predicted values. These results support the concept that parenchymal cells make the largest contribution to remnant uptake *in vivo*. Evidence from the present studies and liver-perfusion studies (Lippielo *et al.*, 1981) suggests that the uptake of remnant lipids by endothelial cells is minimal. Although Kupffer cells take up more remnant lipid per cell than do hepatocytes (Fig. 2), their total overall contribution to remnant uptake *in situ* is less (Lippielo *et al.*, 1981) because there are 10 times more hepatocytes in the liver (Knook & Sleyster, 1980).

The amounts of material taken up by the cells *in vitro* are less than would be predicted from liver-perfusion experiments (Cooper & Yu, 1978; Sherrill & Dietschy, 1978). This was also observed with cultured hepatocytes, which were found to have a decreased capacity for remnant-cholesterol-ester uptake relative to whole liver (Nilsson *et al.*, 1981). The maximum uptake of total cholesterol by the liver after injections of chylomicra is about 2 mg/h per 10 g of liver (Andersen *et al.*, 1977). From the data of Fig. 2 and the mass composition of the remnants it can be calculated that the total uptake of remnant cholesterol by all three cell types is approx. 0.7 μg /h. On the basis of the relative numbers of each cell type in the liver (Knook & Sleyster, 1980), this predicts a total uptake capacity of about 0.6 mg/h per 10 g of liver, only 30% of the actual liver capacity.

We also found that the percentage of [^{14}C]oleoyl lipids taken up by all of the cells was higher than that of the [^3H]lipids, phospholipid and cholesterol. This was true for both PHP and LPL remnants. Since this additional [^{14}C]lipid must be material in excess of what is associated with bound remnants, it is likely that it is taken up by adsorption from particles in the medium. This is not due solely, however, to fatty-acid and partial-triacylglycerol components associated with remnants, because the [^{14}C]triacylglycerol fraction in the post-incubation fluids was equally depleted. This could be due to an acylglycerol hydrolase activity in both parenchymal and non-parenchymal cells. It has been suggested that this enzyme is synthesized and secreted by hepatocytes and becomes bound to non-parenchymal cells (Jansen *et al.*, 1979). It should be noted that remnants incubated without cells in the presence of albumin showed little change in composition (results not shown).

Hepatocytes and Kupffer cells both demonstrate a capacity for remnant-lipid metabolism, as evidenced by changes in the isotopic ratios of esterified to non-esterified cholesterol and radiolabelled methylphospholipid to acylphospholipid (Table 2). This agrees with observations from liver-perfusion studies (Lippielo *et al.*, 1981). Although exchange of non-esterified cholesterol and phospholipid between remnants and cells cannot be ruled out, it probably does not occur to a significant extent. For example, the endothelial cells showed no change in any

of the isotopic ratios, despite significant lipid uptake (cf. Fig. 2 and Table 3). It is unlikely that the exchange of both phospholipid and cholesterol would occur exclusively with Kupffer cells and hepatocytes. This is further supported by the fact that the total cholesterol/phospholipid ratio remains constant with time for all three cell types (Table 3). The only evidence for cholesterol-ester hydrolysis, at least over 2 h, was in hepatocytes and Kupffer cells (Table 2). Since endothelial cells have been shown to possess the necessary enzymic activity (Knook & Sleyster, 1980), it may be that the remnant cholesterol ester has not gained access to intracellular hydrolytic enzymes in 2 h.

In general, the results obtained with PHP and LPL remnants were similar, suggesting that the source of remnants does not profoundly affect remnant uptake and metabolism. However, it was found that the uptake of total remnant lipids by hepatocytes was always higher with PHP remnants (Fig. 2). This was also seen in cultured hepatocytes, where the maximal velocity for remnant uptake was higher for remnants prepared with serum than it was for remnants prepared with purified lipase (Nilsson *et al.*, 1981). It may be that remnants prepared with serum contain relatively more apolipoprotein E (Fig. 1; see also Nilsson *et al.*, 1981). This apolipoprotein has been suggested to be the major recognition factor for remnant binding to cellular receptor sites (Windler *et al.*, 1980). The present results suggest that this may not be as critical a factor in determining the uptake and/or metabolism of remnant lipids by non-parenchymal cells (Fig. 2).

Finally, all three cell types appear to be capable of rapid uptake and metabolism of remnant-fatty-acid components, in agreement with previous studies with isolated hepatocytes (El-Maghrabi *et al.*, 1979) and perfused liver (Lippiello *et al.*, 1981). The present results suggest that this may not be related entirely to the content of fatty acid and partial acylglycerols present in the remnant particle or to the apolipoprotein composition.

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