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Comparison of the Metabolism of Chylomicrons and Chylomicron Remnants by the Perfused Liver

By RONALD S. GARDNER and PETER A. MAYES
Department of Physiology and Biochemistry, Royal Veterinary College, London NW1 0TU, U.K.

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1. The hepatic metabolism of chylomicrons and chylomicron remnants was compared after adding approximately equal numbers of each lipoprotein particle to the perfusate of isolated livers. 2. At least 40% of the added remnants were metabolized by the liver, compared with less than 3% for chylomicrons. 3. There was significantly more net removal of labelled remnants than of chylomicrons by the liver. 4. A greater proportion of labelled cholesterol than of labelled triacylglycerol fatty acids was transferred to the liver from each lipoprotein. 5. Cholesteryl esters of remnants were hydrolysed and triacylglycerol fatty acids of remnants were oxidized to CO_2 more extensively than those of chylomicrons. 6. There was greater oxidation of remnant glycerolipid [1-^{14}C]oleate than of glycerolipid [1-^{14}C]palmitate. 7. A large fraction of the fatty acids of remnants, but not of chylomicrons, was transferred to phospholipids, which were released by the liver in a lipoprotein of relative density <1.006. 8. Label from remnants, but not from chylomicrons, was found in lipoproteins of relative density >1.006, which were not released during perfusion but could be flushed out from the liver at the end of perfusion.

Studies *in vivo* on the metabolism of chylomicrons have implicated the liver as a major organ concerned in their removal from the circulation (French & Morris, 1958; Bragdon & Gordon, 1958). In addition, experiments using the perfused rat liver in the presence of heparin demonstrated substantial metabolism of chylomicron triacylglycerol fatty acids (Morris, 1963; Rodbell *et al.*, 1964). However, heparin is an activator of a lipoprotein lipase present in the rat liver (Mayes & Felts, 1968). When heparin was omitted from the perfusate (Felts & Mayes, 1965) only minimal metabolism of chylomicron triacylglycerol fatty acids was found, and it was suggested that chylomicron triacylglycerol was initially hydrolysed by lipoprotein lipase in peripheral capillary beds before the constituent fatty acids became available to the liver. Redgrave (1970) showed that when chylomicrons were injected intravenously into functionally hepatectomized rats, they underwent hydrolysis by lipoprotein lipase in extrahepatic tissues to form chylomicron remnant particles, which accumulated in the circulation. These lipoproteins were relatively rich in cholesteryl esters, though they still contained triacylglycerol as the major lipid component. Chylomicron remnants injected into the intact rat were taken up by the liver to an appreciable extent.

In the perfused liver the uptake of ^{126}I-labelled chylomicron remnants has been described by Noel *et al.* (1975), and Felts *et al.* (1975) have also shown substantial uptake of remnant triacylglycerol. However, little information has been provided about the metabolism of remnants by the liver. In the present paper we have compared the uptake and metabolism of chylomicron remnants with chylomicrons when equal numbers of the particles were present in the perfusate. A preliminary account of this work has been published (Gardner & Mayes, 1976).

Materials and Methods

Production of labelled chylomicrons

Thoracic ducts of male Wistar rats (380–400g) on normal stock diet (Diet 86; E. Dixon Ltd., Ware, Herts., U.K.), were cannulated (Bollman *et al.*, 1948) under Nembutal (Abbot, Queenborough, Kent, U.K.) anaesthesia. The cannulations were carried out in the early afternoon, when the stomach was virtually empty. As chylomicron samples of low mass were required, no extra lipid was fed. [1α,2α-^{3}H]Cholesterol (50μCi) and either [1-^{14}C]oleic acid or [1-^{14}C]palmitic acid (100μCi; The Radiochemical Centre, Amersham, Bucks., U.K.) were dissolved in 0.4ml of 0.03M-KOH, in which 1mg of sodium tauroglycocholate was dissolved. The final pH was 9.0. When the lymph was flowing, and before the body wall was sutured, the radioactive dose was injected through the stomach wall into the lumen of the pyloric region. The rats were placed in restraining cages and the lymph was collected at room temperature (25°C) overnight. The animals were given access to water *ad libitum*. The lymph was layered in

cellulose nitrate tubes (Beckman) under 0.9% NaCl and centrifuged (29min) for 1×10^6 *g*-min in a fixed-angle rotor (43114–120) in an MSE Superspeed 65 centrifuge at 4°C. The top fraction (2cm) was harvested and used the same day either as labelled chylomicrons or as the precursor of chylomicron remnants.

Production of labelled chylomicron remnants

Chylomicrons were prepared as described above, except that 100μCi of [1α,2α-^{3}H]cholesterol and 250μCi of either [1-^{14}C]oleic acid or [1-^{14}C]-palmitic acid were used. Post-absorptive rats (370–400g) were anaesthetized with Nembutal and functionally hepatectomized by ligating all major blood vessels supplying the liver and the gut. Chylomicron remnants were produced by injecting 2.0–2.5ml of labelled chylomicrons into an iliolumbar vein. After 30min as much blood as possible was withdrawn from the aorta and allowed to clot for 30min at 37°C. After centrifugation (10min, 2000*g*), the serum was layered under 0.9% NaCl and centrifuged for 4×10^6 *g*-min as described above. The top 2cm, containing labelled chylomicron remnants, was harvested and used immediately.

Liver perfusions

Livers were perfused with 130ml of defibrinated dialysed whole rat blood, essentially as described by Mayes & Felts (1966). In the present experiments the perfusions were carried out *in situ* and the blood-flow rate was set at 12ml/min, i.e. approx. 1.25ml/g of tissue per min. As it was desired to detect any differences in the rates of fatty acid oxidation, livers from rats deprived of food for 18–24h were used, since they show higher rates of fatty acid oxidation than do livers from fed animals and have minimal rates of secretion of very-low-density lipoprotein (Mayes & Felts, 1967). At zero time the chylomicron or chylomicron-remnant dose (5ml) was added to the perfusate and the perfusion was continued for 90min.

Increased hepatic-vein pressure causes increased trapping of lipoproteins in the space of Disse (D. F. Tierney & P. A. Mayes, unpublished work). To promote exchange of lipoproteins within that space, the pressure on the hepatic vein was increased every 5min by occluding the outflow cannula for 2–3s until there was just perceptible swelling of the liver.

At the end of the perfusion the liver was flushed with 4×20ml of Krebs–Henseleit (1932) bicarbonate buffer. Each volume was collected separately and analysed to determine the lipoprotein species therein and their lipid composition.

Analytical methods

Serum and liver cholesterol was determined with *o*-phthalaldehyde (Rudel & Morris, 1973) and serum and liver phospholipids were measured by direct colorimetric analysis (Raheja *et al.*, 1973). Methods used in determining blood glucose, ketone bodies and lactate, serum non-esterified fatty acids and triacylglycerol, and methods for separating lipids by t.l.c., have been described previously (Topping & Mayes, 1972, 1976). Radioactivity was counted and corrected for efficiency by the method of Felts & Mayes (1967*a*). $^{14}CO_2$ was collected and determined as described by Mayes & Felts (1966).

Agarose-gel chromatography

Agarose columns were prepared by using Bio-Gel A50m (Bio-Rad Laboratories, Richmond, CA, U.S.A.) by the method of Rudel *et al.* (1974). A virtually complete separation of chylomicrons from chylomicron remnants was achieved.

Analysis of the chylomicron-remnant fraction as separated by ultracentrifugation showed that approx. 75% of the triacylglycerol fatty acid radioactivity was in the remnant peak and the remainder was in the chylomicron peak. There were no further minor peaks.

Experimental design

The experimental design consisted of four groups of perfusions, two where palmitate was used to label the triacylglycerol fatty acid in either chylomicrons or chylomicron remnants, and the other two, where oleate replaced the palmitate as label. Cholesterol and cholesteryl esters were labelled with [^{3}H]cholesterol in all four groups.

Statistical analysis of the results was carried out by Student's *t* test.

Results

Concentrations of metabolites in blood

Blood glucose concentrations remained at 4–5μmol/ml throughout the period of perfusion. There were no statistically significant differences between the groups. Serum non-esterified fatty acid concentrations were steady and ranged between 0.2 and 0.4μmol/ml, and acetoacetate and 3-hydroxybutyrate concentrations were both within 0.2–0.4μmol/ml of blood throughout the experimental period in all groups. The ratio [3-hydroxybutyrate]/[acetoacetate] was approx. 1.0, with no statistical differences between groups. A significant difference was detected in blood lactate concentration, the perfusates of the livers infused with chylomicrons containing approx. 0.4–0.7μmol/ml, whereas the concentration was 2–3-fold higher (1.3–2.0μmol/ml) when the livers were infused with chylomicron remnants ($P < 0.01$). These concentrations were constant throughout the perfusion.

Analysis of the chylomicron and chylomicron-remnant lipoprotein lipid showed triacylglycerol to be the major lipid component on a weight basis, accounting for 92.8±2.6% (mean±S.E.M.) in chylo-

microns and 85.6±2.8% in remnants. Esterified and non-esterified cholesterol contributed 5.4±1.6% of the total mass in chylomicrons and 12.4±2.3% in remnants. Phospholipids made up the remaining 1–3% in all groups.

Fate of labelled chylomicrons and chylomicron remnants

Analysis of the radioactive lipoproteins added to the perfusate showed that approx. 92% of the ^{14}C-labelled fatty acid was located in the triacylglycerol fraction of both chylomicrons and chylomicron remnants for [1-^{14}C]palmitate, and 94% for [1-^{14}C]oleate. Over three-quarters of the remaining label was found in phospholipids. Approx. 85% of the ^{3}H-labelled cholesterol was found in cholesteryl ester, with the remainder in non-esterified cholesterol in both chylomicrons and chylomicron remnants.

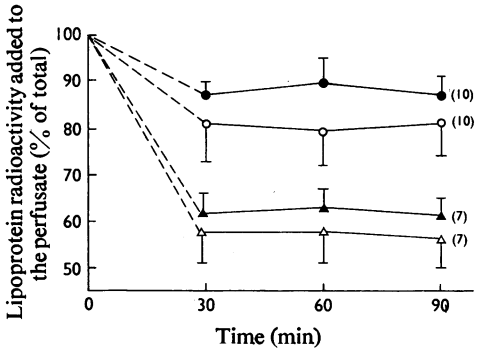

Fig. 1. *Net removal of radioactive cholesterol and fatty acid-labelled chylomicrons and chylomicron remnants from the perfusate during the course of liver perfusions*

The chylomicrons and remnants were labelled with [1,2α-^{3}H]cholesterol and 1-^{14}C-labelled fatty acid. The fatty acid was either palmitic acid or oleic acid. The radioactivity in the chylomicrons added to the perfusate was approx. 1.2 μCi of ^{3}H and 3.5 μCi of ^{14}C and in the remnants was approx. 1.4 μCi of ^{3}H and 4.8 μCi of ^{14}C. The mass of triacylglycerol fatty acid added to the perfusate was 62.8±25.4 (S.D.) μmol for chylomicrons and 23.0±10.7 (S.D.) μmol for remnants. This resulted in approx. 1.2 remnant particles being added for each chylomicron particle added to corresponding perfusions. Total perfusate d <1.006 lipoprotein triacylglycerol fatty acid concentration, i.e. the sum of the lipoproteins added plus that already present in the perfusate, was 2.09±0.51 (S.D.) μmol/ml of perfusate for chylomicrons, and 1.97±0.38 (S.D.) μmol/ml of perfusate for remnants. The differences between fatty acid-labelled chylomicrons and remnants at 30, 60 and 90 min were significant (P<0.001); the differences between cholesterol-labelled chylomicrons and remnants were also significant (P < 0.001) at these time intervals. The numbers of perfusions are shown in parentheses. ●, 1-^{14}C-labelled fatty acid in chylomicrons; ○, [1,2-^{3}H]-cholesterol in chylomicrons; ▲, 1-^{14}C-labelled fatty acid in remnants; △, [1,2-^{3}H]cholesterol in remnants.

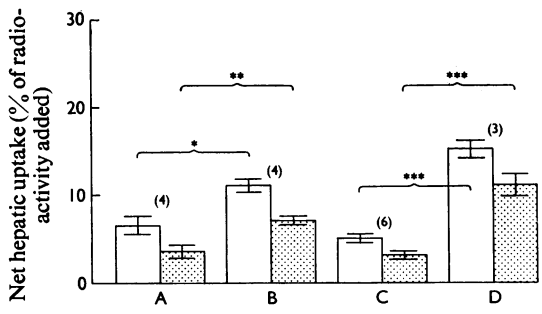

Fig. 2. *Net uptake of radioactive cholesterol and fatty acid-labelled chylomicrons and chylomicron remnants by the perfused liver*

The chylomicrons and chylomicron remnants were labelled with [1α,2α-^{3}H]cholesterol (□) and 1-^{14}C-labelled fatty acid (⁝⁝). The vertical bars indicate S.E.M. The fatty acid was either palmitic acid or oleic acid. Significant differences in the net uptake of cholesterol or fatty acid label between chylomicron and remnant groups after 90 min of perfusion are indicated as follows: *P < 0.05; **P < 0.01; ***P < 0.001. The numbers of perfusions are shown in parentheses. A, [1-^{14}C]Palmitate-labelled chylomicrons; B, [1-^{14}C]palmitate-labelled remnants; C, [1-^{14}C]oleate-labelled chylomicrons; D, [1-^{14}C]-oleate-labelled remnants.

Within the first 30 min after the labelled lipoprotein had been added to the perfusate there was net uptake of radioactive lipid by the liver (Fig. 1), but there was no further change in the radioactivity of acylglycerols or of total cholesterol in the perfusate during the remainder of the perfusion. Irrespective of the type of radioactive label used, the liver removed a greater percentage of the labelled remnants than of chylomicrons from the perfusate (Fig. 2). It was also noted that the liver accumulated a greater percentage of the added [^{3}H]cholesterol radioactivity than of the added triacylglycerol ^{14}C-labelled fatty acid radioactivity, in all four groups of perfusions (Fig. 2).

Oxidation of ^{14}C-labelled fatty acid-labelled chylomicrons and chylomicron remnants

Both the palmitate and oleate in labelled chylomicrons were oxidized to $^{14}CO_2$ to the extent of approx. 0.3% of the total radioactivity added. On the other hand, oxidation of [^{14}C]palmitate-labelled remnants to $^{14}CO_2$ was increased to 0.6% (P < 0.01) and of [^{14}C]oleate-labelled remnants to 1.6% (P < 0.01). The increased oxidation of oleate-labelled remnants compared with palmitate-labelled remnants was statistically significant (P < 0.05).

In the present study the production of ^{14}C-

Table 1. *Metabolism of labelled chylomicrons and chylomicron remnants in perfused livers*

Livers were perfused for 90min with chylomicrons or chylomicron remnants labelled with 1-^{14}C-labelled fatty acid triacylglycerol and [1,2α-^{3}H]cholesteryl ester as described in the text and Fig. 1. Transfer of 1-^{14}C-labelled fatty acid from triacylglycerol to phospholipid was followed by the change in ratio (means ± S.E.M.) shown in columns 1 and 2 from a normalized value of 1.0 for the added lipoprotein. Hydrolysis of esterified [1,2α-^{3}H]cholesteryl ester was followed by the change in ratio shown in columns 3 and 4 from a normalized value of 1.0 for the added lipoprotein. Significant differences from 1.0 are indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. The numbers of perfusions are shown in parentheses.

	Ratio $\frac{\text{1-}^{14}\text{C-labelled fatty acid triacylglycerol}}{\text{1-}^{14}\text{C-labelled fatty acid phospholipid}}$ in:		Ratio $\frac{\text{[1,2}\alpha\text{-}^{3}\text{H]cholesteryl ester}}{\text{[1,2}\alpha\text{-}^{3}\text{H]cholesterol}}$ in:	
	Lipoproteins ($d < 1.006$)	Liver	Lipoproteins ($d < 1.006$)	Liver
Column ...	1	2	3	4
Palmitate-labelled chylomicrons (4)	0.98 ± 0.08	0.17 ± 0.03***	0.88 ± 0.04	0.25 ± 0.03***
Palmitate-labelled remnants (4)	0.33 ± 0.09**	0.10 ± 0.03***	0.67 ± 0.97*	0.14 ± 0.03***
Oleate-labelled chylomicrons (6)	1.01 ± 0.10	0.96 ± 0.10	1.14 ± 0.06	0.12 ± 0.01***
Oleate-labelled remnants (3)	0.37 ± 0.04***	0.26 ± 0.08***	0.71 ± 0.11*	0.12 ± 0.01***

labelled ketone bodies was negligible (0.1%) in all groups. There was no correlation between the mass of triacylglycerol added and the percentage uptake or oxidation in either the chylomicron or the remnant groups.

[^{14}C]Triacylglycerol/^{14}C-labelled phospholipid ratio

In palmitate-labelled chylomicrons and both palmitate- and oleate-labelled remnants, there was a highly significant ($P < 0.001$) decrease in the ratio of ^{14}C present in triacylglycerol to ^{14}C present in phospholipids in the net radioactivity taken up by the liver during 90min, compared with the ratio found in the labelled lipoprotein added to the perfusate (Table 1). For [^{14}C]palmitate-labelled chylomicrons the change in ratio of the radioactivity in the liver accounted for only 2–3% of the total radioactivity added. However, there was no detectable change in this ratio in [^{14}C]oleate-labelled chylomicrons.

There was also a change in the ratio in the d (relative density) < 1.006 lipoprotein lipids of the perfusate in the groups of perfusions with remnants, but not in those with chylomicrons (Table 1). There was no change in ratio when remnants were circulated in blood in the perfusion apparatus but without a liver in the circuit. Thus with the labelled remnants there was a marked interaction with the liver in which some 35–40% of the triacylglycerol fatty acids were converted into phospholipids and the bulk was released into the perfusate in a lipoprotein of d < 1.006 (Fig. 3). The content of phospholipids in the perfusate lipoproteins of $d < 1.006$ did not change significantly during the course of the perfusion with chylomicrons; however, in experiments with remnants there was an increase of approx. 40% (Table 2). These reactions

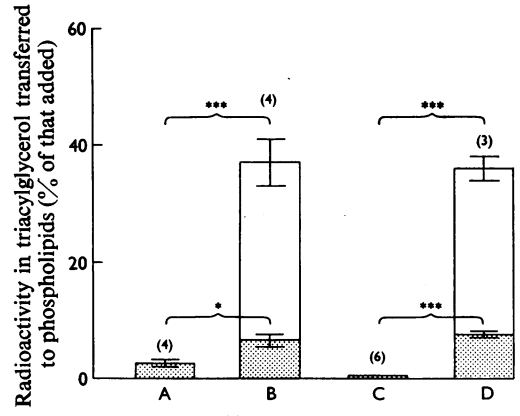

Fig. 3. *Transfer of* 1-^{14}*C-labelled fatty acid from chylomicron and chylomicron-remnant triacylglycerol to phospholipids by the perfused liver*

The transfer of 1-^{14}C-labelled fatty acid from triacylglycerol to phospholipids in experiments with remnants is represented as the sum of the reaction determined in the liver (▨) plus the perfusate lipoproteins ($d < 1.006$) (□) at the end of the perfusion period of 90min. Experiments with chylomicrons are represented by the reaction in the liver only, as the reaction was demonstrated not to have occurred in the perfusate. A, [1-^{14}C]Palmitate-labelled chylomicrons; B, [1-^{14}C]palmitate-labelled remnants; C, [1-^{14}C]oleate-labelled chylomicrons; D, [1-^{14}C]-oleate-labelled remnants. Significant differences between perfusions with chylomicrons and remnants are indicated as follows: $**P < 0.01$; $***P < 0.001$. The amount (μmol) of triacylglycerol fatty acids transferred to phospholipids in groups A, B, C and D was respectively 1.36 ± 0.16 (mean ± S.E.M.), 10.14 ± 0.40, 0.05 ± 0.03 and 6.25 ± 0.69. The numbers of perfusions are shown in parentheses.

Table 2. *Changes in content of phospholipids in lipoproteins of density <1.006 during perfusion of livers with chylomicrons or chylomicron remnants*

Phospholipids were determined at 0 and 90min after perfusion and expressed as the total quantity (means±S.E.M.) in the perfusate after correction for sampling. The numbers of perfusions are indicated in parentheses.

Addition	Content (μmol) 0min	90min	*P* (0–90min)
Chylomicrons (4)	17.0±0.8	18.5±1.1	>0.1
Chylomicron remnants (6)	16.8±1.1	23.4±1.7	<0.01

were not accompanied by release of ^{14}C-labelled non-esterified fatty acids into the perfusate.

Examination of the material that could be flushed out from extracellular spaces in the liver at the termination of the experiment showed that 30–35% of the ^{14}C-labelled fatty acid-labelled lipoprotein recovered was present in the $d > 1.006$ fraction in experiments with remnants, but negligible radioactivity was present in this fraction when chylomicrons were used. However, this fraction comprised only 0.2–0.5% of the added radioactivity in experiments with remnants, and further analysis showed that 60–80% of it was present in the intermediate-density range of lipoproteins ($1.006 < d < 1.019$).

[^{3}H]*Cholesterol-labelled chylomicrons and chylomicron remnants*

The distribution of ^{3}H between esterified and non-esterified cholesterol did not change significantly when chylomicron remnants were produced from labelled chylomicrons in the functionally hepatectomized rat; 82–90% of the total label was in the esterified form in either lipoprotein.

Uptake of labelled total cholesterol by the liver was significantly greater from remnants than from chylomicrons (Fig. 2). Analysis of the $d < 1.006$ serum lipoproteins at the end of the perfusions showed that the [^{3}H]cholesteryl esters: [^{3}H]cholesterol ratio was unchanged from that of the added labelled chylomicrons, but there was a significant decrease in ratio with the labelled remnants, most of the reaction occurring within 30–45min. There was no change in the ratio when the remnants were circulated with blood alone in the absence of a liver. Examination of the liver lipids showed that the [^{3}H]cholesteryl ester: [^{3}H]cholesterol ratio of the lipid taken up from the perfusate was decreased about fivefold compared with the original labelled chylomicrons or remnants added (Table 1). Approx. 4% of the total labelled chylomicron cholesteryl esters were hydrolysed, as against 20–25% for chylomicron remnants (Fig. 4).

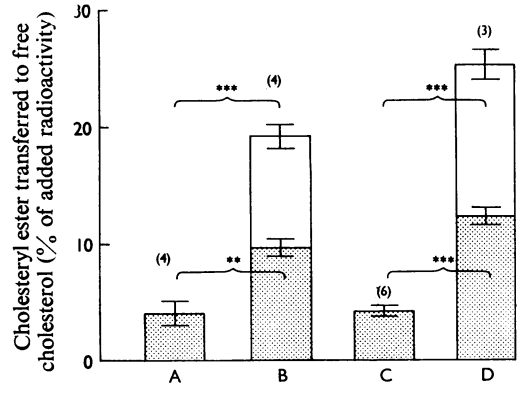

Fig. 4. *Hydrolysis of cholesterol-labelled cholesteryl esters of chylomicrons and chylomicron remnants by the perfused liver*

The hydrolysis of [1α,2α-^{3}H]cholesterol-labelled cholesteryl esters in remnant experiments is represented as the sum of the reaction determined in the liver (▨) plus perfusate lipoproteins $d < 1.006$ (□) at the end of the perfusion period of 90min. Experiments with chylomicrons are represented by the reaction in the liver only, as the reaction was demonstrated not to have occurred in the perfusate. A, [1-^{14}C]-Palmitate-labelled chylomicrons; B, [1-^{14}C]palmitate-labelled remnants; C, [1-^{14}C]oleate-labelled chylomicrons; D, [1-^{14}C]oleate-labelled remnants. Significant differences between perfusions with chylomicrons and remnants are indicated as follows: $*P < 0.05$; $***P < 0.001$. The amount (μmol) of cholesteryl ester hydrolysed in groups A, B, C and D was respectively 0.20±0.05 (mean±S.E.M.), 1.05±0.19, 0.39±0.03 and 1.99±0.02. The numbers of perfusions are shown in parentheses.

Analysis of the lipoproteins washed out of the liver at the termination of the experiment showed that only for chylomicron remnants was there a decrease in the cholesteryl ester: cholesterol ratio. As with the ^{14}C-labelled fatty acid-labelled remnants, approx. 35% of the [^{3}H]cholesterol-labelled lipoprotein washed out was present in the $d > 1.006$ lipoproteins, of which approx. 80% was found in the $1.006 < d < 1.019$ fraction.

Bile

^{3}H-labelled lipid found in the bile accounted for no more than 0.2% of the total radioactivity added, whereas ^{14}C accounted for less than 0.05% of the radioactivity added. No statistically significant differences were found in bile radioactivity between experiments with labelled chylomicrons and chylomicron remnants.

Discussion

Basis of comparison of the metabolism of chylomicron and chylomicron remnants

It has been customary to compare the properties of equal masses of chylomicrons and chylomicron remnants in experiments using the perfused liver (Noel *et al.*, 1975; Felts *et al.*, 1975). An alternative approach is to compare equal numbers of particles of the two lipoproteins, since it would appear that one chylomicron particle gives rise to one remnant particle on reaction with lipoprotein lipase in extrahepatic tissues (Redgrave, 1970). If it is assumed that the diameter of the chylomicron remnant is approximately one-half that of the parent chylomicron (Redgrave, 1970; Mjøs *et al.*, 1975), it can be calculated that if equal masses of the two particles are compared, this represents eight times the number of remnant particles compared with the number of chylomicrons. The average volume (assuming volume is approximately proportional to mass) of the remnants produced from chylomicrons under the present conditions was estimated from the change in ratio of ^{14}C to ^{3}H during remnant formation, the assumption being made that lipoprotein lipase only released the ^{14}C-labelled triacylglycerol fatty acids and not the ^{3}H-labelled cholesterol (Mjøs *et al.*, 1975). From this estimate it was calculated that the average number of particles in the remnant fraction added to the perfusate was actually 1.2 times the number of chylomicrons added.

To avoid any effects due to saturating the hepatic uptake mechanisms, all the experiments were carried out with low masses of chylomicrons and remnants. Thus the chylomicrons and remnant triacylglycerol fatty acids added were approx. 15% and 11% respectively of the total perfusate triacylglycerol fatty acids present in $d < 1.006$ lipoproteins. Within the limits set in the present experiments there was no effect of the mass added on the percentage uptake or metabolism of each of the lipoproteins investigated. Therefore the effects described can be attributed to fundamental differences in properties between chylomicrons and their remnants.

Comparison of the uptake of chylomicrons and chylomicron remnants by the perfused liver

Uptake of lipoprotein particles by the liver does not necessarily indicate that they have been incorporated intracellularly or metabolized. It has been shown both in the perfused liver (Felts, 1965) and *in vivo* (Belfrage, 1966) that chylomicrons that have been taken up by the liver may be washed out again from extracellular spaces. Subsequently it was shown that the degree of extracellular trapping of chylomicrons in sinusoids and the space of Disse may be experimentally increased in the perfused liver, merely by increasing the pressure in the hepatic vein. It was also demonstrated that trapping of chylomicrons by the perfused liver is greater when serum-free perfusates are used (D. F. Tierney & P. A. Mayes, unpublished work). The latter observation has also been made with respect to remnants (R. S. Gardner & P. A. Mayes, unpublished work) and to the uptake of chylomicrons by isolated hepatocytes (Felts & Berry, 1970).

Noel *et al.* (1975) used a plasma-free perfusate and obtained approx. 90% uptake of remnants. Felts *et al.* (1975) used defibrinated rat blood diluted to 20% with Krebs & Henseleit (1932) bicarbonate buffer and obtained 40% uptake in a single pass of perfusate through the liver. With defibrinated whole blood we obtained a net uptake of remnants of approx. 15% in 30min. However, we encouraged the expulsion of particles from the extracellular spaces by systematically raising and releasing the pressure in the hepatic-vein outflow. Thus, owing to variations both in perfusate composition and to undefined and variable hepatic vein pressure, it is difficult to comment on the significance of differences in uptake of chylomicron remnants found by us compared with the results of either Noel *et al.* (1975) or Felts *et al.* (1975). A further point of difference in our results, discussed below, was that the bulk of the remnants that reacted with the liver were released into the perfusate as a modified lipoprotein, rather than being retained by the liver. Nevertheless, a common feature that we have confirmed is the finding that chylomicron remnants are taken up by the perfused liver to a greater extent than are chylomicrons.

Net removal of the lipoprotein particles was maximal within 30min. Thereafter, results based on radioactivity in the perfusate indicated that the liver did not remove any further net mass. Subsequent experiments (R. S. Gardner & P. A. Mayes, unpublished work) with a second dose of chylomicrons or remnants labelled with a different isotope and added at 30min after the first addition have shown uptake into the liver, demonstrating that exchange of lipoprotein is taking place between the liver and the perfusate at these later times.

Metabolic fate of 1-^{14}C-labelled fatty acid-labelled triacylglycerol in chylomicrons and chylomicron remnants

The limited oxidation of chylomicron triacylglycerol fatty acid was in agreement with previously published results (Felts & Mayes, 1965). Remnant triacylglycerol fatty acid was not only oxidized to a significantly greater extent, but oleate-labelled remnants were shown to have increased oxidation compared with palmitate-labelled remnants. This latter result is in keeping with studies showing the greater oxidation of unsaturated fatty acids compared with saturated fatty acids (Nestel & Steinberg, 1963).

The transfer of 1-^{14}C-labelled fatty acid from

triacylglycerol to phospholipids represents the metabolism of a major fraction of the remnant triacylglycerol but only a minor fraction of that of the chylomicron. The ^{14}C radioactivity remaining in the liver after the organ was washed out showed a marked increase in the ratio of ^{14}C-labelled phospholipid to ^{14}C-labelled triacylglycerol, but not with oleate-labelled chylomicrons. The lack of change in ratio with oleate-labelled chylomicrons could be due merely to the lack of reactivity of this particular fatty acid. However, against this hypothesis was the fact that oxidation to $^{14}CO_2$ was similar in magnitude to that obtained with palmitate as tracer. Previous experiments (P. A. Mayes, unpublished work) have shown that at low concentrations, more [1-^{14}C]-palmitate, as non-esterified fatty acid, is esterified to phospholipids compared with non-esterified [1-^{14}C]-oleate. Therefore, it could be that if only a small amount of hydrolysis of labelled chylomicron triacylglycerol takes place, it would be detected more readily as radioactivity transferred into phospholipids when palmitate, rather than oleate, was used as the label. Differences in positional specificity of oleate and palmitate in the acylglycerols might also account for differences in their metabolism.

Only with chylomicron remnants was there an increase in the ^{14}C-labelled phospholipid: [^{14}C]-triacylglycerol ratio in the lipids of $d < 1.006$ lipoproteins. There was no change in the corresponding lipoprotein fraction in chylomicrons. As there was no change in the ratio in remnants when circulated in the perfusate in the absence of a liver, it appears that contact between the liver and the remnants was necessary for the ratio to change. One possible explanation is that labelled triacylglycerol fatty acids were taken up preferentially by the liver, leaving the labelled fatty acids in phospholipids, thus shifting the ratio in the $d < 1.006$ lipoproteins in favour of phospholipids. However, although some preferential uptake cannot be excluded, the total radioactivity in ^{14}C-labelled phospholipids increased approx. tenfold, indicating that a net transfer had taken place. We suggest therefore that reaction with the liver caused the transfer of labelled fatty acids from remnant triacylglycerol into phospholipids, which were subsequently released from the liver in a lipoprotein of different composition from the remnant but which was still in the $d < 1.006$ range.

It was noted that only in experiments with remnants was there a significant increase in phospholipid concentration in the $d < 1.006$ lipoprotein fraction. A tenfold increase in ^{14}C-labelled phospholipid would account for approx. 97% of the increase in mass of phospholipid observed. It would appear that the increased phospholipid phosphorus could only have been derived from the liver during contact with the remnants, as there was no change in phospholipid concentration in the lipoproteins of $d > 1.006$.

The enzyme that brings about liberation of ^{14}C-labelled fatty acid before its oxidation to $^{14}CO_2$ or its transfer from remnant triacylglycerol to phospholipid has not been identified. It is noteworthy that a triacylglycerol hydrolase, similar in some respects to lipoprotein lipase, can be released from the perfused rat liver when high concentrations of heparin are present (Felts & Mayes, 1967*b*). Although the exact location of this enzyme is unknown, it does have similar properties to an enzyme that may be released by heparin from rat hepatic plasma membranes (Assmann *et al.*, 1973). In such a superficial location it may be regarded as a potential candidate for the enzyme that attacks ester bonds of remnant triacylglycerols. Since there was no release of labelled non-esterified fatty acids into the perfusate, and production of $^{14}CO_2$ was diminished compared with what it would be with an infusion of ^{14}C-labelled non-esterified fatty acid, the major reaction involving transfer of labelled fatty acids to phospholipids may have been catalysed by an acyltransferase rather than a hydrolase. Waite & Sisson (1973, 1976) have suggested that phospholipids act as acyl donors in plasma membranes of rat liver. It is possible that *in vivo* the reverse reaction takes place, lysophospholipids acting as acyl acceptors from acylglycerols of remnants.

Metabolic fate of [1,2-^3H]cholesteryl esters in chylomicrons and chylomicron remnants

There was an extensive hydrolysis of cholesteryl ester of chylomicron remnants, but only one-fifth as much hydrolysis in chylomicrons. Again only in experiments on remnants were the metabolic changes in the liver reflected by corresponding changes in the lipoproteins of $d < 1.006$, which was consistent with the liver converting the remnant into a new lipoprotein. A cholesteryl ester hydrolase has been located at or near the cell surface of hepatic parenchymal cells (Stein & Stein, 1969; Riddle *et al.*, 1975), which may be instrumental in carrying out the hydrolysis.

Formation of new lipoproteins from chylomicron remnants

The present work has demonstrated that remnants, but not chylomicrons, give rise to significant quantities of new lipoprotein particles on reaction with the perfused liver. It has been shown that a lipoprotein of $d < 1.006$ accumulates in the perfusate during the course of the perfusion, having an increased phospholipid: triacylglycerol ratio and an increased non-esterified cholesterol: cholesteryl ester ratio. This lipoprotein would appear to be an intermediate particle in remnant metabolism, and represents an end stage in its hepatic metabolism, as no further changes in composition were detectable during the latter half of the perfusion. As it has not been identified in normal rat plasma, this may indicate that it is

normally metabolized rapidly in extrahepatic tissues. However, it is noteworthy that newly secreted very-low-density lipoproteins ($d < 1.006$) contain considerably less phospholipid than is present in very-low-density lipoproteins isolated from normal plasma (Kompiang *et al.*, 1976). This may be due to the normal plasma $d < 1.006$ lipoprotein fraction also containing the phospholipid-rich lipoprotein derived from the metabolism of remnants in the liver.

Previous investigators (Noel *et al.*, 1975; Felts *et al.*, 1975) did not observe any release from the perfused liver of lipoprotein products of hepatic metabolism of chylomicron remnants. Several reasons can be advanced to account for this. In the present experiments, active exchange of material in and out of the space of Disse was encouraged. In the experiments of Noel *et al.* (1975) the absence of serum might allow the lipoproteins to adhere once inside the liver. Also, there were no erythrocytes present in the experiments of Noel *et al.* (1975) and there was only 20% of the normal concentration present in the experiments of Felts *et al.* (1975). This would lead to much lower O_2 consumption and to changes in intracellular redox compared with the present investigations using whole blood (see Mayes & Felts, 1976). Our results could be explained if the release of the new lipoprotein or even the transfer reaction shifting remnant fatty acids into phospholipids were energy-requiring and therefore dependent on adequate oxygenation of the liver.

There was no evidence for a lipoprotein of $d > 1.006$, i.e. a low-density lipoprotein, accumulating in the perfusate, in agreement with both Felts *et al.* (1975) and Noel *et al.* (1975). However, we were able to wash out of the liver a lipoprotein of $d > 1.006$ at the end of the perfusion. The significance of this is unclear, as it accounted for only 0.2–0.6% of the added dose, and may indicate that direct conversion of remnants into a higher-density lipoprotein is not a major process at this particular stage of remnant catabolism.

If the new lipoprotein of $d < 1.006$, which appears to be a major end-product of remnant metabolism in the present system, were degraded in extrahepatic tissues, higher-density lipoproteins might be an end-product of this further degradation.

Total reactivity of chylomicron remnants in the perfused liver

It has been pointed out that uptake of lipoprotein particles by the perfused liver is no criterion of their metabolism by this organ. In the present experiments, metabolism can be assessed by totalling the amount of the original radioactivity in triacylglycerol fatty acids that has been converted into phospholipids and CO_2. This amounts to $37.4 \pm 6.1\%$ (S.E.M.) for [^{14}C]palmitate- and $37.9 \pm 1.6\%$ for [^{14}C]oleate-labelled remnants. The corresponding estimates for palmitate- and oleate-labelled chylomicrons were $2.7 \pm 0.4\%$ and $0.4 \pm 0.2\%$ respectively, demonstrating clearly the increased reactivity of remnants when compared with chylomicrons on a particle-for-particle basis. For remnants this is an underestimate of their reactivity, since analysis on the agarose column showed that the remnant fraction was contaminated with chylomicrons to the extent of approx. 25%.

The present investigations comparing the metabolism of chylomicrons and chylomicron remnants on a particle-for-particle basis have demonstrated significant qualitative and quantitative differences in their lipid metabolism. The major qualitative difference was the large-scale conversion of remnants into a phospholipid-rich lipoprotein of $d < 1.006$, which was released into the perfusate. On a much smaller scale, a lipoprotein of $d > 1.006$ was also formed, which was not released into the perfusate. Quantitatively, remnants differed from chylomicrons in showing a much greater total reactivity in the liver, including greater net uptake, greater oxidation to CO_2, greater conversion of triacylglycerol fatty acid into phospholipids and a greater hydrolysis of cholesteryl esters to non-esterified cholesterol.

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References

Assmann, G., Krauss, R. M., Fredrickson, D. S. & Levy, R. I. (1973) *J. Biol. Chem.* **248**, 1992–1999

Belfrage, P. (1966) *Biochim. Biophys. Acta* **125**, 474–484

Bollman, J. L., Cain, J. C. & Grindlay, J. H. (1948) *J. Lab. Clin. Med.* **33**, 1349–1352

Bragdon, J. H. & Gordon, R. S. (1958) *J. Clin. Invest.* **37**, 574–578

Felts, J. M. (1965) *Ann. N.Y. Acad. Sci.* **131**, 24–33

Felts, J. M. & Berry, M. N. (1970) *Biochim. Biophys. Acta* **231**, 1–7

Felts, J. M. & Mayes, P. A. (1965) *Nature (London)* **206**, 195–196

Felts, J. M. & Mayes, P. A. (1967*a*) *Biochem. J.* **105**, 735–739

Felts, J. M. & Mayes, P. A. (1967*b*) *Nature (London)* **214**, 620–621

Felts, J. M., Itakura, H. & Crane, R. T. (1975) *Biochem. Biophys. Res. Commun.* **66**, 1467–1475

French, J. E. & Morris, B. (1958) *J. Physiol. (London)* **140**, 262–271

Gardner, R. S. & Mayes, P. A. (1976) *Biochem. Soc. Trans.* **4**, 715–716

Kompiang, I. P., Bensadoun, A. & Yang, M. W. W. (1976) *J. Lipid Res.* **17**, 498–505

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's. Z. Physiol. Chem.* **210**, 33–66

Mayes, P. A. & Felts, J. M. (1966) *Proc. Eur. Soc. Study Drug Toxicity* **7**, 16–29

Mayes, P. A. & Felts, J. M. (1967) *Biochem. J.* **105**, 18c–20c

Mayes, P. A. & Felts, J. M. (1968) *Biochem. J.* **108**, 483–487
Mayes, P. A. & Felts, J. M. (1976) *Biochem. J.* **156**, 685–689
Mjøs, O. D., Faergman, O., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 603–615
Morris, B. (1963) *J. Physiol. (London)* **168**, 564–583
Nestel, P. J. & Steinberg, D. (1963) *J. Lipid Res.* **4**, 461–469
Noel, S.-P., Dolphin, P. J. & Rubinstein, D. (1975) *Biochem. Biophys. Res. Commun.* **63**, 764–772
Raheja, R. K., Kaur, C., Singh, A. & Bhatia, I. S. (1973) *J. Lipid Res.* **14**, 695–697
Redgrave, T. G. (1970) *J. Clin. Invest.* **49**, 465–471
Riddle, M. C., Smuckler, E. A. & Glomset, J. A. (1975) *Biochim. Biophys. Acta* **388**, 339–348
Rodbell, M., Scow, R. O. & Chernick, S. S. (1964) *J. Biol. Chem.* **239**, 385–391
Rudel, L. L. & Morris, M. D. (1973) *J. Lipid Res.* **14**, 364–366
Rudel, L. L., Lee, J. A., Morris, M. D. & Felts, J. M. (1974) *Biochem. J.* **139**, 89–95
Stein, O. & Stein, Y. (1969) *Lab. Invest.* **17**, 436–446
Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311
Topping, D. L. & Mayes, P. A. (1976) *Br. J. Nutr.* **36**, 113–126
Waite, M. & Sisson, P. (1973) *J. Biol. Chem.* **248**, 7985–7992
Waite, M. & Sisson, P. (1976) *Biochim. Biophys. Acta* **450**, 301–310