The removal of partially metabolized very-low-density lipoproteins by the perfused rat liver

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1. Donor perfused rat livers were used to prepare VLD (very-low-density) lipoproteins, labelled in their triacylglycerol and protein components with [$1\text{-}^{14}\text{C}$]oleic acid and L-[4,5- ^3H]leucine respectively. Partially metabolized VLD lipoproteins, similarly labelled, were obtained from supradiaphragmatic rats injected with the parent VLD lipoproteins. 2. The triacylglycerol and protein components of the partially metabolized VLD lipoproteins were removed by recipient perfused rat livers at rates much higher than those of the parent VLD lipoproteins. No degradation of the partially metabolized VLD lipoproteins to LD (low-density) lipoproteins occurred during the perfusions. 3. Removal of hepatic lipase from the livers did not significantly affect the rate of removal of the partially metabolized VLD lipoproteins.

The entry of chylomicrons and VLD lipoproteins into the bloodstream from the intestine and liver is followed by the hydrolysis of their constituent triacylglycerols. The hydrolysis is due to the action of lipoprotein lipase at the luminal surfaces of the capillary endothelial cells of the extrahepatic tissues and serves to facilitate the removal of triacylglycerol fatty acids from the circulation (Robinson, 1970).

The progressive loss of triacylglycerol from the chylomicrons and VLD lipoproteins by lipoprotein lipase action requires a restructuring of these lipoprotein complexes. This involves the transfer of their surface protein, phospholipid and cholesterol to the circulating HD lipoproteins and their acquisition, by transfer from the HD lipoproteins, of cholesterol esters formed by the action of phosphatidylcholine:cholesterol acyltransferase in the plasma (Chajek & Fielding, 1978). Eventually such changes can lead to the genesis within the plasma compartment of complexes with the density of the LD lipoproteins (Suri *et al.*, 1978, 1979; Chajek & Eisenberg, 1978; Deckelbaum *et al.*, 1979). However, in the intact animal, the lipoprotein intermediates in the degradation of chylomicrons by lipoprotein lipase action, termed remnants, can also be taken up by the liver and, in the rat at least, hepatic uptake, rather than degradation to LD lipoproteins, appears to be their major fate (Redgrave, 1970; Noel *et al.*, 1975; Felts *et al.*,

1975; Cooper & Yu, 1978; Sherrill & Dietschy, 1978). Indirect evidence for the hepatic uptake of the corresponding intermediates in the degradation of VLD lipoproteins has been provided by Faergeman & Havel (1975) and Faergeman *et al.* (1975), and has been subsequently directly demonstrated in a study published while the present work was in progress (Windler *et al.*, 1980).

The liver contains a lipase (hepatic lipase) that has been implicated in the hepatic uptake of the partial degradation products of chylomicron metabolism (Felts *et al.*, 1975). This lipase is similar in some respects to the lipoprotein lipase of the extrahepatic tissues in that it is released from the liver by heparin (La Rosa *et al.*, 1972; Krauss *et al.*, 1973; Jansen *et al.*, 1977) and is probably located and active at the surfaces of the endothelial cells of the liver sinusoids (Jansen *et al.*, 1978; Kuusi *et al.*, 1979b), in the same way that lipoprotein lipase is released from and is active in the capillary beds of the extrahepatic tissues. However, unlike lipoprotein lipase, the hepatic lipase has no requirement for activator peptides for the expression of activity against the triacylglycerols of its lipoprotein substrates and is resistant to inhibition by strong salt solutions (La Rosa *et al.*, 1972; Krauss *et al.*, 1973).

In the present paper we confirm the capacity of the perfused rat liver to take up the partial degradation products of VLD-lipoprotein metabolism by the extrahepatic tissues (Windler *et al.*, 1980) and show that the triacylglycerol and protein components thereof are removed to similar extents. Moreover, this uptake is shown not to be sig-

Abbreviations used: VLD lipoproteins, very-low-density lipoproteins ($d < 1.006$); LD lipoproteins, low-density lipoproteins ($d 1.006\text{--}1.063$); HD lipoproteins, high-density lipoproteins ($d 1.063\text{--}1.21$).

nificantly affected by the removal from the liver of most of its heparin-releasable lipase activity.

Materials and methods

Materials

The L-[4,5-³H]leucine (130–190 Ci/mmol), [1-¹⁴C]oleic acid (56 mCi/mmol) and the n-[1-¹⁴C]- and n-[1,2(n)-³H]-hexadecane reference standards were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Heparin solution (5000 units/ml) was purchased from Evans Medical Ltd., Speke, Liverpool, U.K. Polyethylene glycol (mol.wt. 6000) was supplied by Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. The sources of all other materials used were as specified by Riley & Robinson (1974) and Suri *et al.* (1979).

Animals

The strain, source and maintenance diet of the rats used in the studies reported were as specified previously (Suri *et al.*, 1979). For the work on the production of VLD lipoproteins by the perfused liver, the rats weighed between 320 and 350 g, and for the studies on lipoprotein removal by the perfused liver, they weighed between 140 and 150 g. Animals weighing between 340 and 380 g were used for the supradiaphragmatic preparations. All the rats used were in the fed state and those providing livers for the synthesis of VLD lipoproteins had their drinking water replaced by 10% (w/v) fructose solution 24 h before the experiment.

Liver perfusions for the production of radioactively labelled VLD lipoproteins

Liver perfusions for VLD-lipoprotein production were carried out in a recycling system as previously described, with washed rat erythrocytes, albumin, glucose and an amino acid mixture in the perfusion medium (Suri *et al.*, 1979). After the flow rate through the liver had stabilized, 0.3 ml of L-[4,5-³H]leucine and 5 ml of an oleic acid–albumin complex containing 1.5 μ Ci of [1-¹⁴C]oleic acid/ml were added to the medium. The oleic acid–albumin complex was then infused at a rate of 1.5 ml/h. The perfusion was carried out for 3.5 h. In some experiments L-[4,5-³H]leucine was omitted from the perfusion medium.

Production of partially metabolized VLD lipoproteins

Supradiaphragmatic rats, prepared by the method of Bezman-Tarcher & Robinson (1965), were injected with radioactively labelled VLD lipoproteins (0.2–0.4 ml) into the left jugular vein. For each experiment, 30 supradiaphragmatic rats were used and they were injected with the total VLD lipoproteins produced by the perfusion of four rat

livers. The initial mean plasma triacylglycerol concentration was 160 mg/100 ml and, assuming that the mean plasma volume of the supradiaphragmatic rat is 28% of that of the whole animal (Suri *et al.*, 1979), the amount of VLD lipoprotein injected was calculated to be sufficient to raise this by 45 mg/100 ml. The supradiaphragmatic rats were bled after 40 min via the abdominal aorta at a time when it was calculated that the plasma triacylglycerol concentration would have fallen by at least 50% (see Suri *et al.*, 1978). The blood obtained from the supradiaphragmatic rats was pooled for the isolation of the partially metabolized VLD lipoproteins of $d < 1.006$.

Liver perfusions for studies on removal of radioactively labelled VLD lipoproteins and partially metabolized VLD lipoproteins

A recycling liver-perfusion system as described by Suri *et al.* (1979) was used, but the perfusion volume was decreased to between 35 ml and 40 ml and the oleic acid–albumin complex, the amino acid mixture and the radioactive leucine were omitted from the medium. The decrease in the volume of the perfusion medium was achieved by the use of a smaller lung and volume reservoir and by the shortening of all connective tubing. The gassed perfusion medium was circulated through the liver for 5 min. Radioactively labelled VLD lipoproteins or partially metabolized VLD lipoproteins (see above) were then added and the perfusion was continued for 10 min. At the end of this time, 5 ml of 0.9% NaCl solution was circulated through the liver via the portal-vein cannula. This cleared the liver of erythrocytes and of unbound lipoprotein complexes, and the evenness of clearance of the liver lobes provided an indication of how well the livers had been perfused. The perfusion medium and the salt solution used for the clearance of the liver were then pooled for the separation of lipoprotein fractions of $d < 1.006$. The livers were weighed, and the mean liver weight ($\pm$ s.d.) was 7.3 ± 0.9 g.

In each experiment, samples of the VLD lipoprotein and partially metabolized VLD-lipoprotein preparations were also perfused for 10 min without livers in the perfusion system. The lipoproteins of $d < 1.006$ were then re-isolated and the recoveries of labelled triacylglycerol and protein were used to correct the values obtained with livers present.

Isolation of lipoproteins

VLD lipoproteins and partially metabolized VLD lipoproteins were isolated from perfusates and from plasma by flotation in the ultracentrifuge at $d < 1.006$ (Suri *et al.*, 1979). The isolated lipoproteins were exhaustively dialysed against 0.9% NaCl solution and concentrated by dry dialysis against polyethylene glycol before further use. Delipidation

of the lipoproteins was carried out by the method of Scanu & Edelstein (1971), and gel filtration of their apoproteins was performed as described by Suri *et al.* (1979).

In some experiments LD lipoproteins (d 1.006–1.063) and HD lipoproteins (d 1.063–1.21) were also isolated from perfusates as described by Suri *et al.* (1979).

Removal of hepatic lipase by perfusion of livers with heparin and the preparation of acetone/ether-dried powders from liver homogenates

Livers were cleared of blood with about 80 ml of 0.9% NaCl solution that was gassed with O_2/CO_2 (19:1). They were then perfused with gassed 0.9% NaCl solution containing heparin (20 units/ml) in single-pass perfusions at a rate of 10–12 ml/min. The perfusates were collected in 2.5 ml fractions for the assay of hepatic lipase, and the livers were finally flushed free of heparin by the perfusion of 60 ml of 0.9% NaCl solution.

In some experiments the livers were then connected to the recycling perfusion system for studies of the removal of partially metabolized VLD lipoproteins. In others, they were homogenized in 0.9% NaCl solution (2 ml/g of liver) and the homogenates were added to 10 vol. of acetone at $-10^\circ C$ and stirred for 30 min. The protein precipitates were filtered through Whatman no. 1 paper at room temperature and the precipitates washed with 100 ml of acetone, followed by 100 ml of ether. They were then dried in a desiccator under vacuum for 30 min and stored at $-70^\circ C$ for up to 24 h. Acetone/ether-dried preparations from livers that had not been perfused with heparin were also prepared in the same way.

Assay of hepatic lipase

Hepatic lipase was assayed by a modification of the procedure of Riley & Robinson (1974) for the assay of lipoprotein lipase with Intralipid (Vitrum, Stockholm) as the triacylglycerol substrate. Serum was omitted from the assay medium, the volume was decreased to 1 ml and the incubations were carried out at $30^\circ C$. One unit of enzyme activity is equivalent to the release of 1 μ mol of fatty acid/h.

Results

Removal of partially metabolized VLD lipoproteins by the perfused rat liver

Fig. 1 shows the results of experiments designed to study the removal by the perfused rat liver of the triacylglycerol and protein components of partially metabolized VLD lipoproteins recovered from the circulations of supradiaphragmatic rats. Three separate lipoprotein preparations were used and a total of eight perfusions were performed.

Triacylglycerol removal

Fig. 1(a) shows that the removal of the ^{14}C -labelled triacylglycerol was linear with respect to its concentration in the perfusion medium over the range of concentrations studied [correlation coefficient (r) = 0.98]. The mean ($\pm$ S.E.M.) total removal of ^{14}C by the whole liver during the 10 min perfusion was $52 \pm 3\%$ of the total ^{14}C radioactivity initially present in the perfusion medium.

The lipoprotein preparations contained ^{14}C -labelled triacylglycerols, derived from the VLD lipoproteins that had been injected into the supradiaphragmatic rats, and unlabelled triacylglycerols, derived from endogenous chylomicrons and VLD lipoproteins present in the bloodstead of the supradiaphragmatic rats at the time of their isolation. The total concentration of lipoprotein triacylglycerol (injected and endogenous) in the perfusion medium in the experiments of Fig. 1(a) ranged between 50 and 260 μ g/ml, and the concentration of the labelled triacylglycerol, as calculated from the known specific radioactivity of the triacylglycerols of the injected VLD lipoproteins, ranged between 20 and 75 μ g/ml. The total concentration was deliberately chosen as being well below the triacylglycerol concentration of rat plasma (1.6 mg/ml in the present work) in order to exclude the likelihood of saturation of the liver receptor sites for triacylglycerol removal (see the Discussion section).

In the experiments of Fig. 1(a), the removal of the partially metabolized VLD-lipoprotein triacylglycerol was measured after a 10 min period of perfusion. Table 1 shows that, at the three different lipoprotein concentrations examined, the removal was progressive over this time period and that the rate of removal declined as the perfusate concentration fell.

Protein removal

The removal by the perfused livers of the 3H -labelled protein of the partially metabolized VLD lipoproteins is shown in Fig. 1(b). Removal of the protein radioactivity, like that of the triacylglycerol radioactivity, was linear with respect to its concentration in the perfusion medium ($r = 0.93$). Moreover, the mean ($\pm$ S.E.M.) total removal of 3H by the whole liver during the 10 min perfusion was $48 \pm 3\%$ of the total 3H radioactivity initially present in the perfusion medium. By the paired t test, this is not significantly different from the percentage removal of [^{14}C]triacylglycerol radioactivity.

The removal of protein by the perfused liver could have been due to the removal of particular apoprotein components of the partially metabolized VLD lipoproteins. However, gel filtration of the lipoprotein apoproteins, after the perfusion of the lipoproteins through the liver, showed that the percentage distribution of radioactivity among the

Fig. 1. *Removal of partially metabolized VLD-lipoprotein triacylglycerol and protein by the perfused rat liver* Supradiaphragmatic rat preparations were injected with VLD lipoproteins that had been labelled in their triacylglycerol and protein components with [$1\text{-}^{14}\text{C}$]oleic acid and L-[4,5- ^3H]leucine respectively. After 40 min, the preparations were exsanguinated. Partially metabolized VLD lipoproteins were then separated and perfused through the vascular beds of isolated rat livers (see the Materials and methods section). Fig. 1(a) shows the removal by the livers of the triacylglycerol radioactivity of the injected partially metabolized VLD lipoproteins as a function of the triacylglycerol radioactivity in the perfusion medium. Fig. 1(b) shows the removal of the protein radioactivity of the same partially metabolized VLD lipoproteins as a function of the protein radioactivity in the perfusion medium. Each point on the graph represents a separate liver perfusion.

Table 1. *Time course of removal of partially metabolized VLD lipoprotein by the perfused rat liver*

Partially metabolized VLD lipoproteins, labelled in their triacylglycerol moiety with [$1\text{-}^{14}\text{C}$]oleic acid, were prepared (see the Materials and methods section) and perfused through three separate livers. In these experiments, the perfusion volume was 40 ml and the flow rate through the liver was 8 ml/min. The concentrations of labelled triacylglycerol, as calculated from the known specific radioactivity (see the text), perfused through the livers were 35, 49 and 67 $\mu\text{g}/\text{ml}$. Perfusate samples (1 ml) were removed at 3, 6, 10, 15 and 20 min. After centrifugation to remove erythrocytes, supernatant radioactivity was determined and the labelled triacylglycerol removed/min per g of liver during successive time intervals was calculated.

Time interval (min)	Liver ...	Rate of removal of partially metabolized VLD lipoprotein (μg of labelled triacylglycerol/min per g of liver)		
		1	2	3
0-3		7.3	12.6	15.3
3-6		7.3	12.3	13
6-10		6.2	9	11
10-15		4.4	6.6	9.4
15-20		2.6	4.4	6.8

three major apoprotein peaks (63%, 19% and 16% in peaks I, II and III; see Suri *et al.*, 1979) was almost identical with that in the same lipoproteins that had been circulated through the perfusion apparatus in the absence of the liver (68%, 18%, 13%).

Distribution of lipoproteins in the medium after liver perfusions

In three of the above experiments, the distribution of triacylglycerol and protein radioactivity in the medium was investigated at the end of the perfusion. Over 95% of the ^{14}C and ^3H remained in the

lipoprotein fraction of $d < 1.006$. Only about 4% was present in the d 1.006–1.063 (LD-lipoprotein) fraction and none could be detected in the d 1.063–1.21 (HD-lipoprotein) or $d > 1.21$ fractions. The 4% of radioactivity that was recovered in the d 1.006–1.063 fraction probably represents incomplete separation of the $d < 1.006$ lipoproteins, since a similar percentage was recovered in the d 1.006–1.063 fraction after perfusions carried out in the absence of the liver. It can be concluded that the partially metabolized VLD lipoproteins that are not removed by the liver are not altered with respect to their density, at least over a 10 min perfusion period.

Removal of unmetabolized VLD lipoproteins by the perfused rat liver

The removal characteristics of the ^{14}C -labelled triacylglycerols of unmetabolized VLD lipoproteins by the perfused rat liver are shown in Fig. 2(a). There is no significant concentration-dependence of the removal process ($r = 0.12$). Moreover, in nine perfusions, the mean ($\pm$ S.E.M.) total removal of ^{14}C by the whole liver during the 10 min perfusion was only $14 \pm 3\%$ of the total ^{14}C radioactivity initially present in the perfusion medium.

A lack of concentration-dependence and a low percentage removal is also observed with respect to the ^3H -labelled protein component of the unmetabolized VLD lipoproteins (Fig. 2b; mean $\pm$ S.E.M. removal of $7 \pm 2\%$). Thus, just as the removal of chylomicrons by the rat liver is much less efficient than that of chylomicron remnants (Redgrave,

1970; Noel *et al.*, 1975; Felts *et al.*, 1975; Cooper, 1977; Andersen *et al.*, 1977; Sherrill & Dietschy, 1978), so it appears is the removal of VLD lipoproteins less efficient than that of their partially metabolized products.

Before the VLD lipoproteins used in the experiments of Fig. 2 were perfused, they were incubated in rat plasma for 40 min at 37°C . This was to ensure that the apoprotein exchange and acquisition that follows the entry of VLD lipoproteins into the bloodstream had taken place (Lewis, 1977; Osborne & Brewer, 1977; Schaefer *et al.*, 1978). They were then recovered by ultracentrifugation and dispersed in the perfusion medium. Particular attention was paid to the choice of the concentrations of lipoproteins in the perfusion medium. One possibility would have been to cover the range of triacylglycerol concentrations used in the experiments of Fig. 1. However, because the unmetabolized VLD lipoproteins have a much higher triacylglycerol content than the partially metabolized VLD lipoproteins, this would have meant that the numbers of lipoprotein particles circulating through the liver would have been considerably lower. Since there is strong evidence that uptake by the liver involves the removal of intact lipoprotein particles (see the Discussion section), achievement of equivalence of particle numbers seemed more important if legitimate comparisons of the uptake of unmetabolized and partially metabolized VLD lipoproteins were to be made.

In practice, therefore, in these experiments the

Fig. 2. Removal of unmetabolized VLD lipoprotein triacylglycerol and protein by the perfused rat liver VLD lipoproteins that had been labelled in their triacylglycerol and protein components with [^{14}C]oleic acid and L-[4,5- ^3H]leucine respectively were incubated for 40 min at 37°C with rat plasma. The VLD lipoproteins were then re-isolated and perfused through the vascular beds of isolated rat livers. Fig. 2(a) shows the removal by the livers of the triacylglycerol radioactivity of the VLD lipoproteins as a function of the triacylglycerol radioactivity in the perfusion medium. Fig. 2(b) shows the removal of the protein radioactivity of the same VLD lipoproteins as a function of the protein radioactivity in the perfusion medium. Each point on the graph represents a separate liver perfusion.

Table 2. Removal of partially metabolized VLD-lipoprotein triacylglycerol and protein by control and hepatic-lipase-depleted livers

Partially metabolized VLD lipoproteins, labelled in their triacylglycerols and proteins with $[1-^{14}\text{C}]$ oleic acid and $\text{L-[4,5-}^3\text{H}]$ leucine respectively, were prepared as described in the legend to Fig. 1. Equivalent concentrations were perfused for 10 min through nine livers that had been depleted of hepatic lipase by pre-perfusion with heparin and through nine control livers that had not been pre-perfused with heparin (see the text). The percentage of added triacylglycerol and protein radioactivity removed/g of liver was calculated. Mean ($\pm$ S.E.M.) removal values are given. The values for the removal of triacylglycerol and protein for the control and test livers are not significantly different on the basis of a non-paired t test.

Liver	Percentage of added triacylglycerol radioactivity removed/g of liver	Percentage of added protein radioactivity removed/g of liver
Control	6.8 ± 0.4	6.0 ± 0.4
Hepatic-lipase-depleted	7.4 ± 0.7	6.6 ± 0.7

Fig. 3. Release of hepatic lipase by heparin

A rat liver was cleared of blood and perfused with 50 ml of heparin in 0.9% NaCl solution (20 units/ml) in a single-pass perfusion system. Hepatic lipase was assayed in successive fractions (2.5 ml) of the perfusate and a typical elution profile for the enzyme is shown.

ments of Fig. 1. This should have achieved approximately the same numbers of lipoprotein particles in the perfusion medium as in those experiments, since the only assumptions made are that the injected and the endogenous VLD lipoproteins are treated equivalently in the supradiaphragmatic rat and that there is no marked movement of partially metabolized VLD-lipoprotein particles out of the $d < 1.006$ range during their circulation. In the light of our previous studies (Suri *et al.*, 1979), such movement is unlikely to amount to more than 15% of the particle numbers injected.

Removal of partially metabolized VLD lipoproteins by perfused rat livers depleted of hepatic lipase

A typical elution profile of hepatic lipase after the perfusion of livers with heparin is shown in Fig. 3. High enzyme activity is present in the perfusion medium immediately after the introduction of heparin, but thereafter there is a rapid decline to a very low activity. Assays of the residual activity of the enzyme in the liver after three such perfusions yielded hepatic lipase activities of 2.4 ± 0.1 units/g of liver, as opposed to values of 8.3 ± 1 units/g for livers not perfused with heparin. It appears therefore that approx. 75% of the total enzyme is released during such heparin perfusions, which is in good agreement with values reported in the literature (Jansen *et al.*, 1977).

The ability of hepatic-lipase-depleted livers to remove partially metabolized VLD lipoproteins perfused through them is compared with that of control livers in Table 2. There is no significant difference between the means for either triacylglycerol or protein removal.

Discussion

Hepatic removal of triacylglycerol-rich lipoproteins

The characteristics of the removal of partially metabolized chylomicrons (remnants) by the rat

VLD lipoproteins were preincubated in a volume of plasma such that their concentration therein was equivalent to that achieved initially in the circulation of the supradiaphragmatic rat after their injection in the experiments of Fig. 1. They were then recovered, concentrated and redispersed in the perfusion medium precisely as were the partially metabolized VLD lipoproteins used in the experi-

liver have been extensively studied. The process is progressive with time and appears to involve the uptake of intact lipoprotein complexes (Floren & Nilsson, 1977; Sherrill & Dietschy, 1978; Cooper & Yu, 1978). The results reported here establish that the removal of partially metabolized VLD lipoproteins by the perfused rat liver shows very similar characteristics. Thus it is also time-dependent and, since the core triacylglycerol and the surface proteins of the lipoproteins are removed at similar rates, appears to involve the uptake of intact complexes. Finally, just as the removal of chylomicron remnants by the perfused rat liver is much more efficient than the removal of their parent chylomicrons, so the removal of partially metabolized VLD lipoproteins is more efficient than the removal of the parent VLD lipoproteins.

The liver-perfusion studies reported here were carried out at low concentrations of partially metabolized VLD lipoproteins in the perfusion medium. This was a deliberate choice, since the lipoprotein preparations were derived from the circulation of supradiaphragmatic rats. They therefore contained not only the radioactively labelled lipoproteins whose removal by the liver was being monitored, but also unlabelled endogenous lipoprotein species of both hepatic and intestinal origin. Since these might compete for the same receptor sites as the labelled species, it was decided to work at concentrations that were likely to be well below those needed to saturate the removal mechanism. We also paid particular attention in planning the present studies to the perfusate lipoprotein concentrations employed in the comparisons of the rates of removal of the partially metabolized VLD lipoproteins with those of the parent VLD lipoproteins. In similar studies comparing chylomicron and chylomicron-remnant removal, equivalent perfusate concentrations of lipoprotein triacylglycerol have frequently been employed (Noel *et al.*, 1975; Felts *et al.*, 1975). However, since the process of removal is probably receptor-mediated, we consider it more appropriate to approximate to equivalent numbers of lipoprotein complexes in the perfusion medium, and the comparisons in the present study were carried out under such conditions. Our observation that the removal of the parent VLD lipoproteins is much slower than that of their partial metabolic products is in agreement with the studies by Windler *et al.* (1980). However, neither we nor they found any concentration-dependence of VLD-lipoprotein removal, as has been reported for chylomicrons (Sherrill & Dietschy, 1978; Cooper & Yu, 1978). This may be a real point of difference between the two groups of lipoproteins, but may also be accounted for by the difficulties of measuring small changes in perfusion-medium concentration at low removal rates.

Role of hepatic lipase in the metabolism of triacylglycerol-rich lipoproteins

From the present work, it can be concluded that the heparin-releasable hepatic lipase is unlikely to be involved in the initial removal of partially metabolized VLD lipoproteins by the perfused liver. Thus release of about 75% of the enzyme by prior perfusion with heparin affected neither the amount nor the characteristics of removal of either triacylglycerol or protein. There have been two studies since the present work was initiated in which hepatic lipase was immobilized *in vivo* by the injection of antibody to the enzyme (Kuusi *et al.*, 1979a; Jansen *et al.*, 1980). In both these, inhibition of the enzyme produced no alterations in the plasma concentrations of triacylglycerol-rich lipoproteins in the short term, but among other things it raised the concentration of HD-lipoprotein phospholipid in the blood. These results are entirely consistent with the present studies, and, together with other work showing that the heparin-releasable liver lipase has phospholipase activity (Ehnholm *et al.*, 1975; Jansen *et al.*, 1977; Sundaram *et al.*, 1978; Van Tol *et al.*, 1980), suggest a primary role of the enzyme in the degradation of the plasma HD-lipoprotein phospholipids.

The 25% of the hepatic lipase activity that was not released by heparin under the conditions of the present experiments seems likely to be intracellular. As in the case of the fraction of adipose-tissue lipoprotein lipase that is associated with the fat-cell, it could represent a precursor of the enzyme at the endothelial cell surface (Robinson, 1970). However, we carried out experiments in the course of the present study in which, after the initial perfusion with heparin and the perfusion with partially metabolized VLD lipoproteins, the livers were reperfused with heparin. No release of lipase into the perfusion medium was observed (B. S. Suri, M. E. Targ & D. S. Robinson, unpublished work). It can therefore be concluded that there was no significant movement of enzyme to the endothelial cell surface, at least during the time for which the lipoproteins were perfused.

An alternative possibility is that the lipase activity that is not heparin-releasable is due to a distinct enzyme with an intracellular role. Thus the removal of chylomicron remnants by the liver is followed by their entry into the liver cells, where their cholesterol components influence the endogenous rate of hepatic cholesterol synthesis (Floren & Nilssen, 1977; Cooper, 1977; Erickson *et al.*, 1977), and there is some evidence that the remnant triacylglycerol may also be hydrolysed after its uptake (Green & Webb, 1964; Bergman *et al.*, 1971). Lysosomal liver lipases that could be responsible for this hydrolysis have been described (Teng & Kaplan, 1974; Debeer *et al.*, 1979), but the possibility that the non-heparin-

releasable lipase activity of the liver could be involved cannot entirely be excluded.

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