# Metabolism of two forms of apolipoprotein B of VLDL by rat liver

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**Abstract** Apolipoprotein B (apoB) is composed of metabolically distinct fractions of higher molecular weight ($apoB_h$) and lower molecular weight ($apoB_l$). When $^{125}$I-labeled very low density lipoprotein (VLDL) prepared from recirculating liver perfusates was injected into rats, labeled $apoB_l$ was preferentially removed from the plasma and $apoB_h$ entered low density lipoprotein (LDL). The time-related movement of labeled $apoB_h$ into higher density fractions was independent of that of labeled $apoB_l$. When $^{125}$I-labeled triglyceride-rich lipoprotein (TRL) prepared from sucrose-fed rats was incubated with plasma from rats injected with heparin and then studied in a recirculating liver perfusion, $apoB_l$ was preferentially removed compared to $apoB_h$. Thus, the loss of $apoB_l$ of hepatic VLDL in vivo was similar to the loss of $apoB_l$ of lipase-treated TRL in vitro. In control perfusions where TRL was incubated with heat-treated postheparin plasma, not only was there less initial hepatic clearance of apoB but the early phase of preferential $apoB_l$ removal during 30 min of perfusion was not observed. ApoE removal from perfusates was the same whether or not the TRL had been treated with heparin-releasable lipases. Apoprotein degradation, as indicated by the appearance in the perfusate of labeled degradation products, occurred 30 min after the preferential phase of $apoB_l$ removal. These results suggest that hepatic clearance of VLDL and TRL remnants is favored by lipolysis and by the presence of $apoB_l$ on the particle that enhances their hepatic binding and degradation.—**Sparks, C. E., D. J. Rader, and J. B. Marsh.** Metabolism of two forms of apolipoprotein B of VLDL by rat liver. *J. Lipid Res.* 1983. **24:** 156–166.

**Supplementary key words** very low density lipoprotein • intestinal apoB • triglyceride-rich lipoprotein • hepatic lipoprotein metabolism

The triglyceride-rich lipoprotein (TRL) includes chylomicrons and very low density lipoprotein (VLDL) of intestinal and hepatic origin. TRL contains apoB as a structural or nonexchangeable protein, integral to the particle (1). In the plasma, TRL forms an overlapping system of particles with regard to size, density, lipid and protein composition. When TRL enters the plasma, TRL remnants are formed by the action of lipoprotein lipase (2). Following a series of enzymatic and exchange reactions, some TRL remnants become low density lipoprotein (LDL) while others are cleared by the liver. In rats, chylomicron remnants are cleared by the liver (3–5) and in humans they are assumed to follow a similar pathway. VLDL, on the other hand, follows a precursor-product relationship with LDL in humans (6, 7), whereas in rats the majority of VLDL remnants are hepatically cleared (8). Factors affecting the divergent paths of TRL catabolism await clarification, but TRL particle composition has been reasoned to influence hepatic clearance, thus inversely affecting the amount converted to LDL. It has been demonstrated that the exchangeable apolipoproteins also affect hepatic clearance of TRL remnants with apoC inhibiting clearance (9, 10) while apoE facilitates remnant removal (9–13). Recently it was discovered that apoB can exist in two forms (14, 15), raising the possibility that the form of apoB determines whether TRL remnants are rapidly cleared by the liver or whether they enter a slower catabolic pathway as part of LDL ultimately to be taken up by hepatic and peripheral cells (16).

Both forms of apoB are present in human and rat TRL (14, 15). In humans, Kane, Hardman, and Paulus (14) have designated the higher and lower molecular weight forms as B-100 and B-48, respectively. ApoB-100 is associated with VLDL and LDL and apoB-48 is associated with chylomicrons (14). ApoB-100 and apoB-48 differ slightly in amino acid composition (14). Two forms of apoB were first reported in the rat by Krishnaiah et al. (15). We have designated them as $apoB_h$ (apoB of higher molecular weight) and $apoB_l$ (apoB of lower molecular weight) (17). $ApoB_h$ and $apoB_l$ have similar amino acid composition (15, 17) but, as in humans, there are subtle immunologic differences (15, 18)

Abbreviations: TRL, triglyceride-rich lipoprotein ($d < 1.006$ g/ml containing intestinal lipoprotein); VLDL, very low density lipoprotein ($d < 1.006$ g/ml of hepatic origin); IDL, intermediate density lipoprotein ($1.006 < d < 1.02$ g/ml); LDL, low density lipoprotein ($1.02 < d < 1.06$ g/ml); HDL, high density lipoprotein ($1.06 < d < 1.21$ g/ml); SDS, sodium dodecyl sulfate; $apoB_h$, apolipoprotein B of higher molecular weight; $apoB_l$, apolipoprotein B of lower molecular weight; PHP, postheparin plasma, plasma from animals injected with heparin.

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and differences in peptide sequences (18). In the rat, the intestine synthesizes only $apoB_l$ (15, 18–20), and the liver synthesizes both $apoB_h$ and $apoB_l$ (18, 19, 21, 22). The two forms are metabolically heterogeneous following injection of $^{125}$I-labeled TRL containing both forms. The results of Elovson et al. (18) and from this laboratory (23) have demonstrated that $apoB_l$ is cleared from plasma faster than $apoB_h$ and that $apoB_h$ preferentially enters LDL, where it becomes the dominant form (18, 19). The more rapid turnover of $apoB_l$ in vivo was also shown by Wu and Windmueller (21). We have suggested an independent hepatic binding site for $apoB_l$ (23) based on evidence comparing the clearances from plasma of $apoB_h$, $apoB_l$, apoE, and apoC and their appearance in liver following injection of $^{125}$I-labeled TRL.

The present experiments were undertaken first, to show that earlier in vivo data using $^{125}$I-labeled TRL from plasma of sucrose-fed rats were not due to the presence of lipoproteins of intestinal origin and second, to show that prior lipolysis is required for hepatic discrimination of $apoB_h$ and $apoB_l$. For these purposes, we isolated VLDL from liver perfusates, labeled it with $^{125}$I, and followed the in vivo removal of labeled apoproteins after intravenous injection into normal rats. Simultaneously, we measured the appearance of apoprotein label in lipoproteins of different density classes. Additionally, we incubated $^{125}$I-labeled TRL from sucrose-fed rats with plasma from rats injected with heparin (PHP) and studied apoprotein clearance in recirculating liver perfusions, using heat-inactivated PHP-incubated-TRL as a control. The results confirm and extend our earlier observations of the metabolic heterogeneity of apoB in the rat and they suggest the existence of separate hepatic pathways for $apoB_h$ and $apoB_l$ in terms of binding, uptake, and degradation.

## MATERIALS AND METHODS

### Preparation of TRL

Lipoproteins were isolated from the sera of male rats of the Fischer 344 strain weighing 200–300 g. The rats were fed for 5–10 days on a diet containing 68% (w/w) sucrose, 10% (w/w) vegetable oil, and 18% (w/w) casein (ICN, Nutritional Biochemicals, Cleveland, OH). The rats were lightly anesthetized by intraperitoneal injection of 30 mg/kg of nembutal and blood was obtained by aortic puncture at 9–11 AM from animals fed ad libitum. The TRL fraction ($d < 1.006$ g/ml) contained both chylomicrons and VLDL and was prepared by ultracentrifugation of pooled sera as previously described (17, 23).

### Preparation of hepatic VLDL

All rats were 200–300 g males of the Fischer 344 strain. The rats were fed laboratory chow ad libitum for at least 2 weeks prior to their use in liver perfusion. Rats were anesthetized by intraperitoneal injection of 60 mg/kg of nembutal and livers were removed and perfused with buffer outside the system for 3 min to remove blood. Liver perfusions were carried out as described previously (24–26) except that the outflow cannula drained into the inflow reservoir (recirculating perfusion). The system was washed prior to perfusion by 1% (w/v) Triton X-100 and rinsed thoroughly with deionized water prior to the addition of buffer. After the liver was incorporated, perfusions were carried out at 37°C using a perfusate volume of 75 ml at a flow rate of 25 ml/min. The apparatus included 5 feet of silastic tubing (27) in a flask which allowed equilibrium of the perfusion medium with 95% $O_2$/5% $CO_2$. The perfusion medium consisted of 0.1% (w/v) glucose in Krebs-Ringer bicarbonate. Control studies indicated adequate oxygen uptake by the liver. At the end of 60 min of perfusion, the perfusate was collected and hepatic VLDL was isolated by ultracentrifugation at $d < 1.006$ g/ml (24–26).

### Preparation of $^{125}$I-labeled TRL and nascent hepatic $^{125}$I-labeled very low density lipoprotein

At a concentration of 2–4 mg/ml protein, measured by the method of Lowry et al. (28), $^{125}$I-TRL and nascent hepatic $^{125}$I-VLDL were prepared employing the McFarlane-ICl method (29) using the modification of Bilheimer, Eisenberg, and Levy (30). The $^{125}$I-TRL and $^{125}$I-VLDL had less than one iodine atom per molecule of apoprotein assuming an apoprotein molecular weight of $2 \times 10^5$. Specific activities ranged from 49–125 dpm/ng protein (mean ± SD, 85 ± 38 dpm/ng) and percent lipid label ranged from 25–38% (mean ± SD, 31 ± 7%).

### Preparation of remnant $^{125}$I-TRL

Postheparin plasma (PHP) was prepared by injecting rats under ether anesthesia intravenously with heparin giving a calculated plasma concentration of 10 U/ml. Blood was obtained by aortic puncture 10 min later and plasma was obtained by removal of cells by centrifugation. Remnant $^{125}$I-TRL was prepared by incubating $^{125}$I-TRL (1–2 mg of protein/ml) with an equal volume of PHP for 30 min at 37°C according to the method of Eisenberg and Rachmilewitz (31). Control $^{125}$I-TRL was prepared by incubating $^{125}$I-TRL with an equal volume of PHP previously heated at 56°C for 30 min to inactivate lipase. The PHP was kept at 4°C prior to incubation with TRL and was used within the hour after collection. As a control for the TRL incubations, he-



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patic $^{125}$I-VLDL was added to heated PHP (control VLDL) and PHP (VLDL remnant) and following incubation, the VLDL, IDL, and LDL were isolated by sequential density ultracentrifugation using our modification (26, 32) of the method of Havel, Eder, and Bragdon (33). In control VLDL, the distribution of the total $apoB_h$ or $apoB_l$ radioactivity present in the $d < 1.06$ g/ml lipoproteins in VLDL, IDL, and LDL was 79%, 16%, and 5%, respectively, of total $apoB_h$ compared to 84%, 13%, and 3% of total $apoB_l$. In VLDL remnants, the comparable distribution of radioactivity in VLDL, IDL, and LDL was 30%, 28%, and 42%, respectively, of the total $apoB_h$ compared to 28%, 29%, and 43% of the total $apoB_l$.

### $^{125}$I-TRL and remnant $^{125}$I-TRL in rat liver perfusion

Perfusions were carried out in a recirculating system as described above for preparation of nascent hepatic VLDL. To the perfusion medium containing 2 mg/ml of albumin (Pentex bovine serum albumin Fraction V, Miles Laboratories, Elkhart, IN) was added control $^{125}$I-TRL or remnant $^{125}$I-TRL (5 $\mu$g of TRL protein/ml perfusion medium). The mixture of TRL plus PHP or heated PHP was added to the perfusate. The final heparin concentration in the perfusate after dilution was less than 0.05 units of heparin per ml. The perfusate (75 ml) was sampled (−10 min) and then recirculated for 10 min through the perfusion apparatus and then sampled again at the start of liver perfusion (0 min). The liver was incorporated into the system and duplicate 1-ml samples of perfusate were collected at 5, 15, 30, and 60 min after the perfusion was begun and were prepared for column analysis immediately afterwards.

### Nascent hepatic $^{125}$I-VLDL injection studies

Rats were 200–300 g males of the Fischer 344 strain and were fed laboratory chow ad libitum. Rats for injection studies were anesthetized by intraperitoneal injection of 30 mg/kg of nembutal and were injected intravenously with hepatic $^{125}$I-VLDL via an exposed saphenous vein. An average of 0.42 ml of 0.15 M NaCl–2 mM EDTA, pH 7.4, containing the VLDL was injected averaging 0.36 mg of protein and $2.6 \times 10^6$ dpm. At designated times following injection, 5 ml of blood was collected from the rat by aortic puncture into a syringe containing 0.2 ml of a solution of 0.2 M EDTA, pH 7.4. Total plasma radioactivity was estimated by determining the label contained in triplicate 0.2-ml plasma samples corrected for dilution with EDTA. The average dpm/ml plasma times the plasma volume, which was assumed to equal 4.2% of the body weight, gave the total plasma radioactivity (34). For radioactivity in plasma lipoprotein fractions, the plasma was first separated by sequential density ultracentrifugation.

### Analysis of $^{125}$I-labeled apoproteins by SDS-gel filtration column chromatography

Whole plasma, perfusion medium, and lipoprotein fractions (VLDL, IDL, and LDL) containing $^{125}$I-labeled apoproteins were prepared for column analysis. Whole plasma (0.2 ml) and lipoprotein fractions (1 ml) were first delipidized as previously described (26, 32) using a modification of the method of Lux, John, and Brewer (35). Delipidized plasma and lipoprotein or 1 ml of perfusion medium were added to 1 ml of MD-buffer and heated for 3–5 min at 100°C. The MD-buffer consisted of 0.1 M Tris (hydroxymethyl) aminomethane, adjusted to pH 7.4, containing 10% (w/v) SDS, 10% (v/v) glycerol, and 10% (v/v) 2-mercaptoethanol. After cooling, the entire sample was applied to $165 \times 1.5$ cm glass columns (Bio-Rad Laboratories, Richmond, CA) containing Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in 0.1 M sodium phosphate buffer, pH 7.4, and 1% (w/v) SDS as described previously (17). Up to 2.0 ml of sample was applied to the column and apoproteins were eluted with phosphate–SDS buffer at an average flow rate of 7–8 ml/hr. Fractions were collected at 30-min intervals and radioassayed. Column recovery was uniformly greater than 95% of the applied radioactivity.

### SDS-polyacrylamide gel electrophoresis

Hepatic $^{125}$I-VLDL was analyzed by SDS-polyacrylamide gel electrophoresis on 5% acrylamide gels as described previously (26, 32) and bands were visualized by Coomassie brilliant blue staining and were identified as indicated in Fig. 1.

### Radioactivity measurements

Radioassays of $^{125}$I-labeled lipoprotein and of column fractions from chromatography of $^{125}$I-labeled apoproteins were made in Searle 1144 gamma scintillation spectrometer with a counting efficiency of 82% for $^{125}$iodine. Counting errors in all cases were kept less than 5%. Student's $t$ test was used to calculate statistical significance and all results are expressed as the mean ± standard deviation (SD).

## RESULTS

### Disappearance of apoB label from plasma following injection of hepatic $^{125}$I-VLDL

The disappearance of apoproteins of hepatic VLDL from plasma was followed after intravenous injection of



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the labeled lipoprotein into the rat. Hepatic VLDL was prepared by ultracentrifugation of medium from recirculating rat liver perfusion and labeled as described in Methods. The apoprotein composition of hepatic VLDL using SDS-polyacrylamide gel electrophoresis is shown

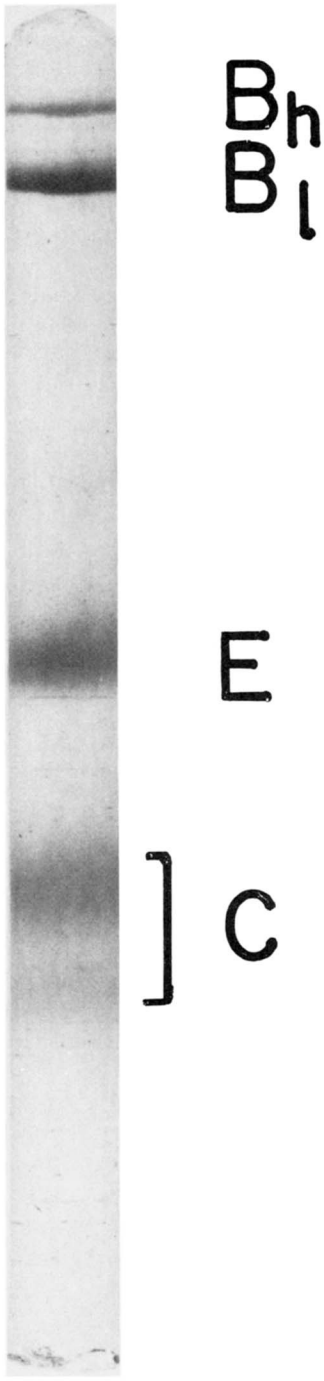


**Fig. 1.** Separation of $^{125}$I-VLDL apoprotein by SDS-polyacrylamide gel electrophoresis on 5% polyacrylamide gels as described in Methods. Apo$B_h$ ($B_h$), apo$B_l$ ($B_l$), apoE (E), and the C apoproteins (C) are clearly separated by this procedure and are indicated next to their corresponding bands.

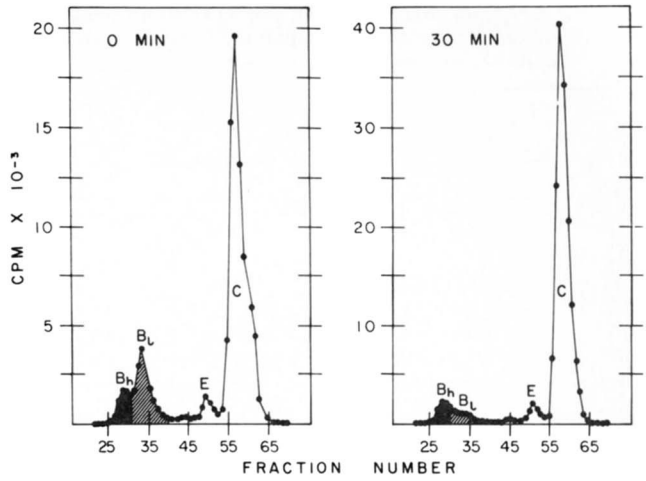


**Fig. 2.** SDS-column chromatography of rat plasma 0 and 30 min after injection of hepatic $^{125}$I-VLDL. For 0 min, $^{125}$I-VLDL was added to rat plasma. The SDS-columns were run as described in Methods. Radioactivity of effluent fractions was plotted against fraction number to give the radioactivity distribution showing four major radioactive apoprotein regions representing apo$B_h$ ($B_h$), apo$B_l$ ($B_l$), apoE (E), and apoC-lipid (C) as indicated.

in **Fig. 1.** The hepatic $^{125}$I-VLDL was injected intravenously and plasma was collected 5, 15, and 30 min thereafter and analyzed by SDS-column chromatography. For the initial time point sample, (0 min), hepatic $^{125}$I-VLDL added to rat plasma was analyzed. The plasma distribution of $^{125}$I-apoprotein radioactivity at 0 min and 30 min is shown in **Fig. 2.** As seen in the figure, after 30 min, the plasma $^{125}$I-apoprotein distribution changed considerably with marked reduction in radioactivity of labeled apo$B_l$. The apo$B_h$ and apo$B_l$ radioactivity as a percent of the total plasma radioactivity at each time point was calculated and the results of the percent distribution in plasma are summarized in **Table 1.** As seen in the table, after 5 min the radioactivity contributed by $^{125}$I-apo$B_l$ to the total plasma radioactivity declined from 14% to 5.7% of the total followed by a slower reduction to 2.5% over the next 25 min. In contrast, $^{125}$I-apo$B_h$ contributed a relatively constant percent to the total plasma radioactivity the entire time. The ratio of radioactivity contributed by labeled apo$B_l$ to that contributed by labeled apo$B_h$ to the total plasma radioactivity changed from 2.4 at 0 min to 0.4 at 30 min, indicating the preferential loss of apo$B_l$ label from plasma. The percent of labeled apo$B_l$ and apo$B_h$ remaining in plasma 5, 15, and 30 min after injection was calculated and the results are also shown in Table 1. After 5 min over two-thirds of apo$B_l$ radioactivity had been cleared from plasma and after 30 min only 12% of the initial dose remained. This contrasted with the clearance of labeled apo$B_h$, where after 5 min one-third of apo$B_h$ radioactivity had been cleared, with no further removal after an additional 25 min.



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TABLE 1. ApoB radioactivity as a percent of total plasma radioactivity and percent of injected apoB radioactivity remaining in plasma following injection of hepatic $^{125}$I-VLDL

| | Percent of Total Plasma Radioactivity[a] | | Percent of Injected Dose Remaining[b] | |
|---|---|---|---|---|
| Time | $ApoB_h$ | $ApoB_l$ | $ApoB_h$ | $ApoB_l$ |
| *min* | | | | |
| 0 | 5.7 ± 0.6 | 14.0 ± 0.7 | 100 | 100 |
| 5 | 4.9 ± 0.6 | 5.7 ± 1.1 | 66.3 ± 10.3 | 30.2 ± 2.1 |
| 15 | 5.4 ± 0.7 | 4.5 ± 1.5 | 74.5 ± 5.9 | 25.3 ± 7.0 |
| 30 | 5.6 ± 0.7 | 2.5 ± 0.4 | 66.8 ± 7.8 | 12.1 ± 2.1 |

[a] The plasma distribution of apoprotein radioactivity was determined by SDS-column chromatography of plasma 5, 15, and 30 min after intravenous injection of hepatic $^{125}$I-VLDL as described in Methods. In the case of 0 min, hepatic $^{125}$I-VLDL was added to rat plasma and then chromatographed. ApoB ($apoB_h$ or $apoB_l$) radioactivity as a percent of total plasma radioactivity was calculated by adding radioactivity of appropriate effluent column fractions and dividing by the total column radioactivity times 100. There were four experiments at each time point and results are expressed as the average ± SD.

[b] The percent of injected apoB ($apoB_h$ or $apoB_l$) radioactivity remaining in plasma after injection of hepatic $^{125}$I-VLDL was calculated as follows. At each time point the assayed total radioactivity per ml of plasma was multiplied by the percent of radioactivity contributed by each apoB as determined by SDS-column analysis, giving the radioactivity of $apoB_h$ or $apoB_l$ per ml plasma at each time point. This value was taken as a percentage of the initial dose of either labeled $apoB_h$ or $apoB_l$ per ml plasma estimated by chromatography of the 0-min plasma and corrected for plasma dilution, assuming the plasma volume was 4.2% of the body weight.

## ApoB label of injected hepatic $^{125}$I-VLDL in isolated plasma lipoprotein fractions

Samples of plasma 0, 5, 15, and 30 min after injection of hepatic $^{125}$I-VLDL were taken for lipoprotein analysis. The plasma was separated into VLDL, IDL, and LDL density classes by sequential density ultracentrifugation and the apoB label of each fraction from a plasma pool of four rats (2 ml plasma/rat) was analyzed by SDS-column chromatography. The apoprotein radioactivity distribution of IDL and LDL as a time course is seen in **Fig. 3** and **Fig. 4,** respectively. In IDL, the peak of $apoB_l$ radioactivity enrichment occurred in the 5-min sample, whereas that of $apoB_h$ was in the 15-min sample, suggesting differences in conversion of VLDL-$apoB_l$- and VLDL-$apoB_h$-containing lipoproteins into IDL. In LDL, the maximum $apoB_l$ radioactivity enrichment occurred at 15 min. No maximum was observed for labeled $apoB_h$ in LDL and labeled $apoB_l$ in IDL. The percent of injected $apoB_h$ and $apoB_l$ radioactivity remaining in plasma (TOTAL) and in plasma VLDL (V), IDL (I) and LDL (L), 5, 15, and 30 min after injection of hepatic $^{125}$I-VLDL was calculated and the results were plotted as a time course as seen in **Fig. 5.** When the two apoB fractions are compared, it can be seen that labeled $apoB_l$ always disappeared from plasma and plasma VLDL more rapidly than labeled $apoB_h$. The most rapid loss was during the first 5 min after

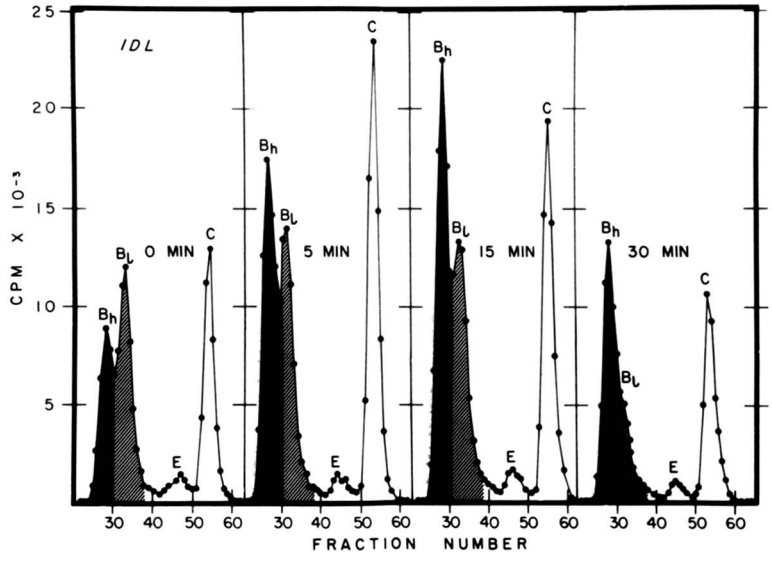


**Fig. 3.** The distribution of $^{125}$I-apoprotein radioactivity of plasma IDL ($1.006 < d < 1.02$ g/ml) after injection of hepatic $^{125}$I-VLDL. Plasma was collected 5, 15, and 30 min after injection and for 0 min, $^{125}$I-VLDL was added to 2 ml of rat plasma to approximate the concentration of radioactivity in the plasma at 0 time. Delipidized lipoprotein fractions containing apoprotein label were mixed with SDS buffer and analyzed by SDS-column chromatography. Radioactivity of effluent fractions was plotted against fraction number to give the radioactivity distribution and $apoB_h$ (shaded) ($B_h$), $apoB_l$ (crosshatched) ($B_l$), apoE (E), and apoC (C) radioactivity are indicated. To allow comparisons of ultracentrifugal fractions, lipoprotein was harvested from an equivalent volume of plasma. The area under each apoprotein peak represents lipoprotein apoprotein radioactivity of 8 ml of plasma (2 ml of plasma pooled from each of four rats).



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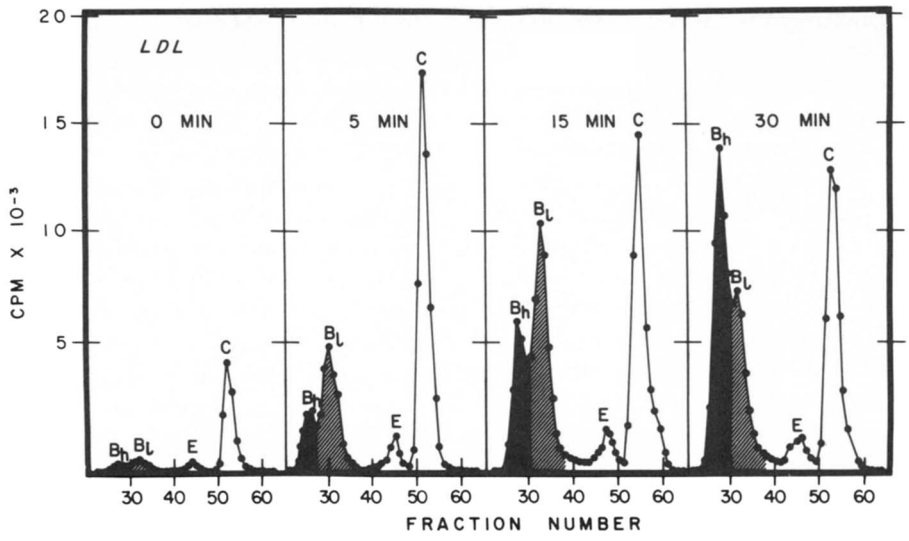


**Fig. 4.** The distribution of $^{125}$I-apoprotein radioactivity of plasma LDL (1.02 < d < 1.06 g/ml) after injection of hepatic $^{125}$I-VLDL. See legend to Fig. 3.

injection. Labeled apo$B_h$ entered IDL from 5 to 15 min and its disappearance from IDL between 15 and 30 min paralleled its entry into LDL.

### Hepatic clearance of control and of lipase-treated $^{125}$I-TRL (remnant) by perfused liver

The hepatic removal of apoproteins of TRL and of lipase-treated (remnant) TRL was studied in recirculating liver perfusion. The TRL was prepared from plasma of sucrose-fed rats and radioiodinated as described in Methods. Control and remnant $^{125}$I-TRL were prepared by preliminary incubation of $^{125}$I-TRL with postheparin plasma or with postheparin plasma heated to destroy lipase activity, respectively. After test lipoprotein was added to the buffer, the perfusate was recirculated through the system for 10 min before incorporation of the isolated liver. The perfusate was sampled (−10 min) and sampled again at the time the liver

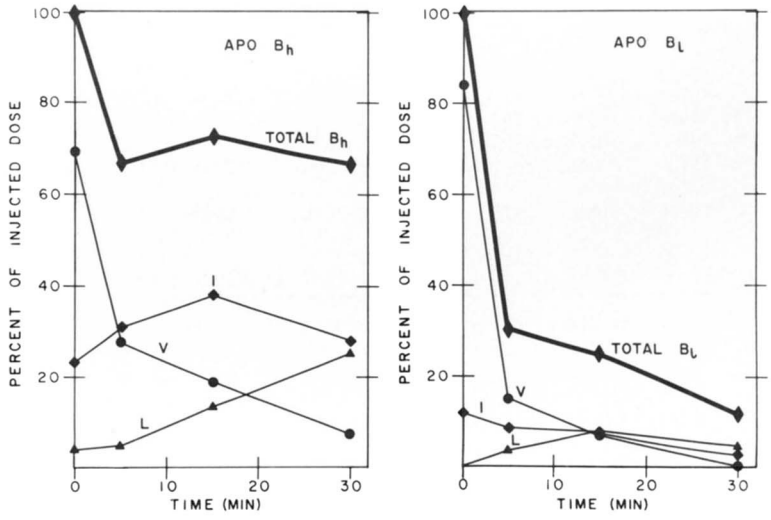


**Fig. 5.** The percent of injected $^{125}$I-apo$B_h$ and $^{125}$I-apo$B_l$ radioactivity remaining in plasma (total, — ♦ —) and remaining in plasma VLDL (V, ● – ● – ●), IDL (I, ♦ – ♦ – ♦), and LDL (L, ▲ – ▲ – ▲) 5, 15, and 30 min after injection of hepatic $^{125}$I-VLDL. The initial injected dose of $^{125}$I-apo$B_h$ and of $^{125}$I-apo$B_l$ was determined by SDS-column chromatography of 0-min plasma and radioassay of the appropriate effluent fractions. The concentration of $^{125}$I-apo$B_h$ and $^{125}$I-apo$B_l$ radioactivity in 1 ml of plasma or of lipoprotein in 1 ml of plasma of timed samples was determined by similar column analyses. The total plasma or lipoprotein radioactivity was calculated by multiplying radioactivity/ml times the plasma volume, assumed to be 4.2% of the body weight. Labeled apo$B_h$ and apo$B_l$ of plasma or lipoprotein divided by the initial injected dose in plasma for each apoB times 100 gave the percent of injected dose remaining.



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was incorporated (0 min) and following 5, 15, 30, and 60 min of liver perfusion. One-ml samples of perfusate were analyzed by SDS-column chromatography and the concentration (dpm/ml perfusate) of each radioactive column region was determined by adding the radioactivity of appropriate effluent fractions. Radioactive regions monitored corresponded to labeled $apoB_h$, $apoB_l$, apoE, apoC plus lipid, and a low molecular weight fraction containing labeled peptides, amino acids, and iodide. In order to compare individual experiments, results were normalized by adjusting initial perfusate total radioactivity (−10 min) to 100,000 dpm/ml and by multiplying other concentrations by the same normalization factor. The calculated results from experiments are summarized in **Table 2.** There was no detectable change in the concentration of radioactivity in any column region of the control or remnant $^{125}$I-TRL during the 10-min pre-perfusion equilibration period (−10 min to 0 min). From the time the liver was incorporated (0 min) until 30 min of perfusion in control $^{125}$I-TRL experiments, there were parallel time-dependent declines in the concentration of radioactivity in $apoB_h$, $apoB_l$, and apoE regions amounting to the loss of roughly one-third from the original perfusate concentration. From 30 to 60 min of perfusion, there was a significant decline in the concentration of $apoB_l$ radioactivity while the labeled low molecular weight fraction almost doubled during the same period. In remnant $^{125}$I-TRL experiments, there were significant declines in the concentration of radioactivity in $apoB_h$ and $apoB_l$ regions at the 5-min time point. The most dramatic decline occurred with $apoB_l$ where more than 40% of the original concentration was removed from the perfusate compared to a 25% loss of labeled $apoB_h$. The loss of labeled $apoB_l$ continued progressively from 5 to 60 min, at which time more than 80% had been removed from the perfusate. There was much slower removal of $apoB_h$ and apoE from 5 to 60 min than of $apoB_l$, and by 60 min more than 50% of each remained in the perfusate. In remnant experiments between 0 and 30 min, the labeled low

TABLE 2. Concentrations of radioactive column regions from chromatography of rat liver perfusates containing added $^{125}$I-TRL[a]

| Region | −10 Min[b] | 0 Min | 5 Min | 15 Min | 30 Min | 60 Min |
|---|---|---|---|---|---|---|
| Control $^{125}$I-TRL | | | | | | |
| $ApoB_h$ | 3364 ± 114 | 3403 ± 170 | 3216 ± 480 | 2557 ± 441 | 2282 ± 356 | 1617 ± 113 |
| $ApoB_l$ | 7853 ± 512 | 7914 ± 509 | 7067 ± 296 | 6410 ± 105 | 5038 ± 468 | 2331 ± 380 |
| ApoE | 3898 ± 46 | 3789 ± 172 | 3354 ± 170 | 2891 ± 161 | 2741 ± 131 | 2452 ± 141 |
| ApoC-lipid | 80386 ± 427 | 79387 ± 786 | 72444 ± 1116 | 70401 ± 1994 | 68601 ± 493 | 63447 ± 3264 |
| Low molecular weight[c] | 2965 ± 918 | 2873 ± 875 | 2952 ± 760 | 3052 ± 627 | 4146 ± 439 | 7778 ± 462 |
| Total column radioactivity[d] | 100,000 | 99043 ± 360 | 90768 ± 1141 | 87007 ± 2511 | 84521 ± 1045 | 79097 ± 3430 |
| Remnant $^{125}$I-TRL | | | | | | |
| $ApoB_h$ | 3054 ± 280 | 3155 ± 126 | 2354 ± 130 | 1774 ± 240 | 1592 ± 114 | 1696 ± 356 |
| $ApoB_l$ | 8251 ± 221 | 8168 ± 253 | 4568 ± 669 | 2804 ± 488 | 1922 ± 425 | 1277 ± 389 |
| ApoE | 4013 ± 346 | 4037 ± 156 | 3160 ± 333 | 2803 ± 271 | 2643 ± 244 | 2254 ± 245 |
| ApoC-lipid | 79905 ± 1192 | 79665 ± 2866 | 62626 ± 3204 | 57811 ± 3192 | 58827 ± 3392 | 58465 ± 4989 |
| Low molecular weight[c] | 3136 ± 790 | 3026 ± 477 | 2670 ± 329 | 3302 ± 424 | 7355 ± 2404 | 14087 ± 2222 |
| Total column radioactivity[d] | 100,000 | 99724 ± 3296 | 76797 ± 4163 | 69919 ± 3600 | 73811 ± 4709 | 79230 ± 5786 |

[a] $^{125}$I-TRL was added to postheparin plasma and the mixture was incubated at 37°C for 30 min. The mixture was then added to the perfusate to be studied (Remnant $^{125}$I-TRL). Control $^{125}$I-TRL was prepared identically by incubation of $^{125}$I-TRL with the same postheparin plasma except that the postheparin plasma was heat-inactivated prior to incubation with $^{125}$I-TRL. One-ml samples of perfusate were prepared and analyzed as described in Methods by SDS-column chromatography and effluent column fractions were radioassayed. The radioactivity of each fraction was plotted against fraction number to give a radioactivity distribution. Five regions were established to correspond to $^{125}$I-$apoB_h$, $^{125}$I-$apoB_l$, $^{125}$I-apoE, $^{125}$I-apoC/$^{125}$I-lipid, and a low molecular weight region which included $^{125}$I-peptides, $^{125}$I-amino acid, and $^{125}$Iodide (17). The concentration of radioactivity per ml of perfusate of each region was the sum of effluent fractions within a specified region. To allow comparisons of concentrations between experiments, the results were normalized to 100,000 dpm per ml of initial (−10 min) perfusate radioactivity. Actual initial perfusate radioactivity averaged $1 \times 10^6$ dpm/ml and average accumulated counts for radioassay were $4.8 \times 10^6$ cpm/ml perfusate. The 0-min, 5-min, 15-min, 30-min, and 60-min regional concentrations were multiplied by the same normalization factor used to adjust the initial perfusate radioactivity to 100,000 dpm/ml. The results in the Table at each time point are expressed as average dpm/ml ± SD. There were three control and four remnant $^{125}$I-TRL experiments.

[b] The perfusate was recirculated through the system for 10 min prior to incorporation of the isolated liver, and was analyzed as described above.

[c] Low molecular weight region included $^{125}$I-peptides, $^{125}$I-amino acid, and $^{125}$Iodide.

[d] Total column radioactivity was the sum of normalized concentrations of radioactivity per ml of perfusate for effluent fractions from the entire column. Differences from 100,000 dpm/ml represent absolute losses from the perfusate to the liver. The initial radioactivity (−10 min) was adjusted to 100,000 dpm/ml mathematically.



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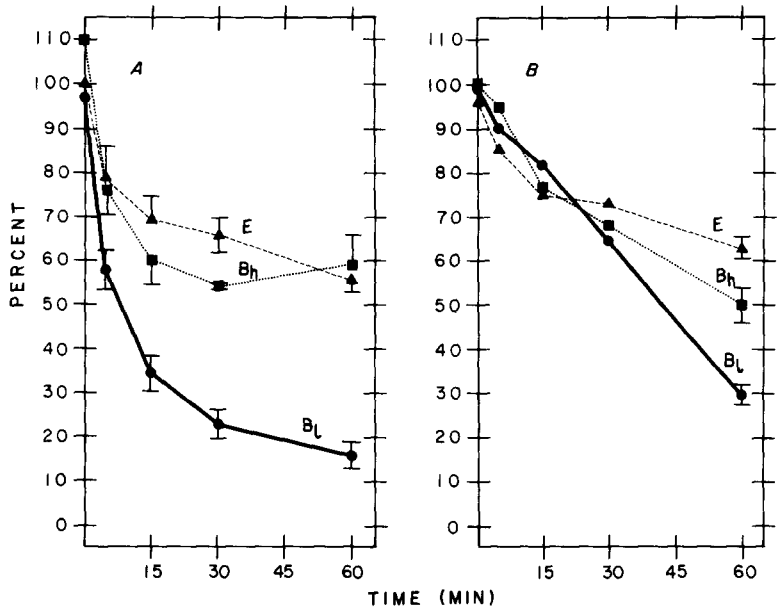


**Fig. 6.** The removal of labeled $apoB_h$ (— ■ —), $apoB_l$ (— ● —), and apoE (— ▲ —) in rat liver perfusion of remnant $^{125}$I-TRL (A) and control $^{125}$I-TRL (B). $^{125}$I-TRL was added to postheparin plasma and the mixture was incubated at 37°C for 30 min. The mixture (remnant $^{125}$I-TRL) was then quantitatively added to the perfusate to be studied (panel A). Control $^{125}$I-TRL experiments (panel B) consisted of identical incubations of $^{125}$I-TRL with postheparin plasma except that the postheparin plasma had been heat-inactivated prior to incubation with $^{125}$I-TRL as described in Methods. The percent of apoprotein radioactivity concentration remaining in the perfusate after addition of $^{125}$I-TRL was calculated as follows. At each time point, 1 ml of perfusate was chromatographed and the radioactivity present in $apoB_h$, $apoB_l$, and apoE column regions was calculated. Apoprotein radioactivity/ml perfusate was multiplied by the perfusate volume, giving the total apoprotein radioactivity, and at each time point this value was expressed as a percentage of the radioactivity present initially (−10 min). A single error bar indicates one SD. The mean bracketed by double error bars is discrete ($P < 0.05$) from mean values at a given time. Means bracketed by single error bars are not discrete from each other at a given time. The absence of error bars indicates that there were no significant differences in mean values of $apoB_h$, $apoB_l$, and apoE at a given time.

molecular weight fraction doubled in concentration, and by 60 min the perfusate contained more than four times the initial concentration.

In **Fig. 6,** results from remnant (panel A) and from control (panel B) $^{125}$I-TRL experiments are presented where $apoB_l$, $apoB_h$, and apoE results are expressed as the percent of initial concentration remaining in the perfusate at each time point. In remnant TRL perfusions there was rapid and preferential removal of $apoB_l$ compared to the other apoproteins. The disappearance curves for $apoB_l$, $apoB_h$, and apoE were similar in control $^{125}$I-TRL experiments for the first 30 min; however, preferential removal of $apoB_l$ was seen from 30 to 60 min. The disappearance curves for labeled apoE were similar in control and remnant TRL perfusions over the entire 60-min perfusion.

## DISCUSSION

Clearance from plasma of injected apoB of hepatic VLDL was studied by analysis of labeled apoproteins in plasma following injection of $^{125}$I-VLDL which was prepared from recirculating rat liver perfusates. In these studies more than 50% of the injected dose of $apoB_l$ label was removed from the plasma by 5 min ($t_{1/2}$, <5 min). This differed from the decay of $apoB_h$ where only one-third of the initial dose was removed after 5 min and very little more was removed after 60 min. The half-life of $apoB_l$ of hepatic VLDL was similar to that reported by us previously for $apoB_l$ of $^{125}$I-TRL from sucrose-fed rats (23). The earlier study also showed preferential hepatic clearance of $apoB_l$ compared to $apoB_h$ (23). The rapid hepatic clearance of $apoB_l$ seen in previous experiments was therefore not a result of contamination of TRL with $apoB_l$ particles of intestinal origin. Elovson et al. (18) and Wu and Windmueller (21) have also indicated the more rapid turnover of $apoB_l$ than $apoB_h$ in rats. At present we do not have an explanation for the rapid and almost complete disappearance of $apoB_h$ from rat VLDL reported by Elovson et al. (18). The plasma $apoB_l$ turnover of hepatic VLDL in the present work and of sucrose-induced TRL in previous studies was similar to that recently reported by Van't Hooft et al. (20) for intestinally



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synthesized chylomicron apoB$_l$ ($t_{1/2}$, 5 min). The similarity of half-lives for apoB$_l$ of hepatic VLDL, of sucrose-induced TRL, and of chylomicrons strongly suggests that the rapid hepatic clearance of apoB$_l$ is a property of the protein independent of its site of synthesis.

About one-third of apoB$_h$ was initially cleared from plasma following injection of hepatic VLDL. In addition, a portion of apoB$_l$ entered LDL, unlike chylomicron-associated apoB$_l$ which is almost entirely cleared prior to entry into LDL (20). We have previously suggested the possibility that there is a population of hepatic VLDL particles that contains both apoB$_h$ and apoB$_l$ (23). If the amount of apoB$_l$ per VLDL particle were a determinant in hepatic clearance, then particles containing both forms of apoB would have clearance rates intermediate to those of particles containing only apoB$_l$ or only apoB$_h$. The rapid clearance of a discrete portion of apoB$_h$ at a rate intermediate to the majority of apoB$_l$ and the majority of apoB$_h$ was observed in these experiments. The slower turnover of particles containing both forms of apoB, with resultant longer plasma residence time, would allow longer contact with endothelial lipases and favor their entry into LDL. This would provide a possible explanation for the difference in entry of hepatic apoB$_l$ into LDL compared to intestinal apoB$_l$. Direct demonstration of VLDL containing both apoB forms will be necessary to confirm this possibility. The majority of apoB$_h$ entered LDL following hepatic VLDL injection. From 15–30 min, apoB$_h$ entry into LDL paralleled its removal from IDL and its appearance in LDL was independent of apoB$_l$. The progressive entry of apoB$_h$ into LDL provides an explanation for the fact that apoB$_h$ is the dominant form of apoB in LDL in rats in terms of mass (18, 19). Thus, in the rat, LDL-associated apoB$_h$ and apoB$_l$ are primarily of hepatic origin.

In order to study the role of the liver in removal of apoB, recirculating liver perfusion studies were performed. In the current study, using $^{125}$I-labeled TRL in recirculating liver perfusion, the role of the liver in removal of apoB$_h$, apoB$_l$, apoE, and apoC plus lipid of TRL was studied. TRL protein concentrations in the range of the reported $K_d$ of chylomicrons (12) were used in the perfusate and the production of low molecular weight products in the perfusate served as a measure of lysosomal degradation of apoprotein (17). TRL was pre-incubated with postheparin plasma (PHP) heated to inactive lipase. The mixture (control TRL) was then added to perfusates and the loss of labeled apoprotein and appearance of degradation products were assayed. Using control TRL perfused for 30 min, there were parallel and linear time-dependent declines in apoB$_h$, apoB$_l$, and apoE amounting to one-third of the initial dose. The lack of preferential binding of either apoB suggests a hepatic binding site for TRL independent of apoB composition. This is supported by a recent study by Borensztajn et al. (36) who demonstrated that chylomicrons artificially free of apoB were hepatically cleared. After 30 min, however, apoB$_l$ was preferentially removed compared to other apoproteins. Delay in selective apoB$_l$ removal suggests that TRL underwent modification, possibly by hepatic lipase, prior to selective apoB$_l$ removal.

TRL remnants were prepared by incubation of TRL with PHP, which results in a shift of TRL-apoB from predominantly VLDL density to IDL and LDL densities and the formation of remnants enriched in apoB relative to apoC (31). When TRL remnants were perfused there was rapid and preferential removal of apoB$_l$ compared to apoB$_h$. Following preferential apoB$_l$ removal, there was evidence of apoprotein degradation. These results support the idea that lipase interaction with TRL is necessary prior to selective and rapid hepatic clearance of apoB$_l$. There was a significant difference in apoB$_h$ removal between remnant and control TRL experiments indicating that lipase interactions with TRL may also facilitate removal of a portion of apoB$_h$.

In TRL remnant perfusions the rapid preferential binding of apoB$_l$ resulted in the appearance of degradation products in the perfusate between 15 and 30 min. In control TRL perfusions, the delay in preferential apoB$_l$ binding was associated with a concomitant delay in the appearance of degradation products (Table 2). These results indicate that preferential binding of apoB$_l$ favors degradation of the bound particles. The observed binding of TRL and TRL remnants in the liver is probably not due to the LDL receptor, as the results of Mahley et al. (37) suggest there is little LDL receptor expression in adult animals. Low numbers of LDL receptors would result in a prolongation of LDL residence time in plasma but does not imply that the LDL receptor in liver is not physiologically important in regulating plasma LDL levels. More than 50% of rat LDL may ultimately be catabolized by the liver in adults (16).

A hepatic receptor for apoE has been demonstrated and characterized in rat liver and similarities between apoE binding and TRL remnant binding have led to the concept that apoE mediates TRL remnant clearance. ApoE present in triglyceride emulsions (9) or apoE enrichment of lipoprotein fractions facilitate hepatic clearance (10–13). Studies by Mahley et al. (37) suggest that hepatic apoE binding is not related to the expression of the classical LDL (apoB-apoE) receptor described by Brown and Goldstein (38). The current studies suggest that the role for apoE in TRL remnant catabolism is more complex than simply mediation of binding, uptake, and lysosomal degradation of asso-



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ciated TRL remnants. ApoE disappearance from perfusates was similar in control and remnant experiments, unlike the marked increase in binding of apoB-enriched remnants following lipase exposure. The studies of Van't Hooft and Havel (39) indicate that apoE can dissociate from TRL following injection and that its subsequent catabolism parallels that of HDL. In addition, apoE of rat HDL is probably catabolized as part of an HDL particle (40) and apoA-I likely mediates this HDL particle catabolism (41), implying that apoE eventually associates with HDL particles containing apoA-I. We suggest that the majority of apoE bound to the liver as part of TRL remnants re-enters the plasma apoE pool after facilitating remnant binding and that most apoE is not internalized along with the $apoB_l$-rich remnant. TRL enriched in $apoB_l$ then favors lysosomal degradation of its associated remnant.

The pathway for hepatic catabolism of TRL remnants apparently involves both apoE and apoB, but the exact role for each has not been fully established. There is little question that apoE is a determinant in TRL remnant recognition. The current study indicates that the composition of the TRL remnant with respect to $apoB_l$ also influences its catabolic fate, especially in terms of lysosomal interaction. The composition of a TRL remnant that is favorable for rapid catabolism is enrichment with $apoB_l$ with respect to $apoB_h$, apoE, and apoC. We suggest that the initial interaction of TRL remnants with liver is facilitated by apoE, but that the later processes of hepatic uptake and degradation are favored by particle enrichment with $apoB_l$. The results suggest a hepatic binding site for $apoB_l$, but an hepatic receptor for $apoB_l$ has not been established. Further research will be required to determine how $apoB_l$ composition affects hepatic catabolism of TRL remnants.

We wish to thank Mrs. Arline Ritz and Mrs. Sara Massey for their excellent technical assistance and Dr. Janet L. DeHoff-Sparks for help in the preparation of the manuscript. This work was supported in part by NIH Grant No. P01-HL-22633.

*Manuscript received 23 February 1982 and in revised form 23 August 1982.*

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