Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver

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Abstract The participation of hepatic lipase in the initial uptake and processing of chylomicron remnants was studied in the isolated, perfused rat liver. Hepatic lipase activity was either reduced by preperfusion of livers with heparin or inhibited with specific rat hepatic lipase antibodies. [^{3}H]palmitate-labeled chylomicron remnants were recirculated through control and treated livers for 15 min; the livers were then flushed, homogenized, and endosome-rich fractions were isolated. Depletion of hepatic lipase activity by both methods reduced the uptake of chylomicron remnants and hydrolysis of their component triglycerides by perfused rat livers, but at the same time significantly increased the rate of endocytosis of those chylomicron remnants taken up. We conclude that hepatic lipase facilitates, but is not essential for, the initial uptake of chylomicron remnants by rat liver. Furthermore, endocytosis of chylomicron remnants does not require binding to hepatic lipase or the associated hydrolysis of remnant lipids.—**Shafi, S., S. E. Brady, A. Bensadoun, and R. J. Havel.** Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J. Lipid Res.* 1994. **35:** 709–720.

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Chylomicron remnants, produced by the action of lipoprotein lipase on chylomicron lipids, are rapidly taken up by the liver. The remnant particles enter hepatocytes by receptor-mediated endocytosis, leading to hydrolysis of component lipids and proteins in lysosomes (1). In earlier research, we obtained evidence that endocytosis of chylomicron remnants is delayed after uptake by the liver from the blood of normal rats (2). By contrast, no such delay was observed in estradiol-treated rats, in which low density lipoprotein (LDL) receptors that can mediate endocytosis of chylomicron remnants had been induced manyfold (3). From these results, we postulated that chylomicron remnants to a substantial extent interact initially with other binding sites on liver cell surfaces, such as heparan sulfate, hepatic lipase, or the LDL receptor-related protein (LRP) (2). We also postulated that additional modifications might take place or be required before endocytosis occurs, such as enzymatic attack by hepatic lipase or acquisition of apolipoprotein E, known to be present on the microvillous surface of hepatocytes (2). These interactions or modifications might be bypassed in the estradiol-treated rat because the extraordinary number of LDL receptors on the microvillous surface would permit direct interaction with a transmembrane protein that can mediate the endocytic event.

Several investigators have provided evidence that hepatic lipase participates in the hepatic uptake of chylomicron remnants (4–7). Furthermore, Brasaemle, Cornely-Moss, and Bensadoun (8) have shown that treatment of chylomicron remnants with hepatic lipase increases the exposure of certain antigenic sites in apoE. In the current study, we have sought to obtain further understanding of the role of hepatic lipase in the uptake and processing of chylomicron remnants in the rat. Our approach has been to evaluate separately the uptake and endocytosis of chylomicron remnants in the isolated, perfused rat liver under conditions in which the activity of hepatic lipase was reduced by prior perfusion with heparin or by exposure of the liver to hepatic lipase antibodies.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (200–240 g), maintained on standard Purina rat chow diet, were used to obtain lymph and prepare livers for perfusions.

Antiserum

Goat antiserum against rat hepatic lipase was purified by affinity chromatography against rat hepatic lipase coupled to Sepharose 4B (9, 10). One mg of the IgG fraction of the antiserum inhibited 440 μmol • h^{-1} of hepatic lipase activity, equivalent to about 44 μg active enzyme.

Abbreviations: LDL, low density lipoprotein(s); LRP, LDL receptor-related protein; apo, apolipoprotein; FFA, free fatty acid.

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Chemicals and radiochemicals

Bovine serum albumin, protease inhibitors (benzamidine, bacitracin, aprotinin, and pepstatin), heparin sodium (1000 USP units/ml) and goat IgG were purchased from Sigma (St. Louis, MO). [^{3}H]palmitic acid, [^{3}H]oleic acid, and [^{3}H]triolein were from New England Nuclear (Wilmington, DE). All the other analytical grade reagents were from Fisher (Santa Clara, CA). Lipid standards were from Matreya Inc. (Pleasant Gap, PA).

Preparation of radiolabeled lymphatic chylomicrons and their remnants

Rat intestinal lymph was collected into disodium EDTA (0.68 μM) gentamycin (0.025 mg/ml, final concentration) at 10°C for 7–9 h during intraduodenal infusion at a rate of 2.5 ml/h of 38 nmol [^{3}H]palmitic acid (74 mBq) and 3.9 μmol unlabeled palmitic acid dispersed in 20 ml 0.15 M NaCl containing bovine serum albumin (4%) and glucose (555 mM) (11). Lymph was layered under Krebs-Henseleit buffer (pH 7.4) without glucose or Ca^{2+} and separated at 12°C by ultracentrifugal flotation at δ = 1.007 g/ml for 3 × 10^7 g_{av} min (12). The recovered chylomicrons were recentrifuged (1.5 × 10^7 g_{av} min) under the same conditions and were used within 10 hours.

Chylomicron remnants were produced in functionally eviscerated rats (280–300 g) maintained under ether anesthesia, by injecting chylomicrons (8–10 mg triglycerides/0.34–0.70 mg total cholesterol) into an exposed femoral vein (12). Chylomicrons were allowed to circulate for 20–35 min to obtain remnants in which 75–90% of the triglycerides had been removed by hydrolysis. Blood was then obtained from the abdominal aorta and the density of the serum was raised to 1.019 g/ml by addition of D_2O/0.15 M NaCl. Remnants were then separated by ultracentrifugation overnight at 12°C (10^8 g_{av} min) and used immediately.

Perfusion of isolated livers

For liver perfusions (13) rats were anesthetized with diethyl ether, and laparotomy was performed. The exposed bile duct, portal vein, and vena cava were cannulated, and the vascular bed was flushed with Krebs-Henseleit buffer containing 10% washed rat erythrocytes and glucose (5.55 mM). The liver was removed, connected to the perfusion system, and allowed to recover for 30 min at 37°C by recycling perfusion of 60 ml buffer containing 12% erythrocytes oxygenated with 95% O_2/5% CO_2 (13). The liver and perfusate were maintained at 37°C throughout the experiment. Two livers (control and experimental) were routinely perfused in parallel at a flow rate of 12 ml • min^{-1}. After the recovery period the liver was flushed with a fresh perfusate for 10 min in a single pass mode. Finally, recirculating perfusion with 60 ml Krebs-Henseleit buffer (12% erythrocytes) containing [^{3}H]palmitate-labeled remnants (1.0 mg triglycerides/0.22–0.38 mg total cholesterol) was reinstituted and perfusion continued for 15 min. A portion of the initial perfusate (1.4–1.6 ml) was taken for the analysis of ^{3}H. Samples were taken from the effluent cannulae after 5, 10, and 15 min, erythrocytes were removed by centrifugation, and the supernatant fluid was taken for extraction of lipids and for analysis of protein and ^{3}H. At the end of the perfusion, livers were thoroughly flushed with ice-cold 0.15 M NaCl (120 ml), blotted, weighed, and homogenized in 0.25 M sucrose containing protease inhibitors [benzamidine (0.3 mg/ml), bacitracin (0.8 mg/ml), aprotinin (10 μg/ml), and pepstatin (10 μg/ml)]. Recovery of ^{3}H-labeled triglycerides and cholesteryl esters estimated from the sum of total perfusate and liver homogenate usually exceeded 95%.

Experiments with heparin

Prior to the addition of radiolabeled chylomicron remnants and after a 20-min recovery period as described above, the liver was depleted of hepatic lipase activity by perfusion for 10 min in a recirculating system with fresh perfusate (10% hematocrit) containing 25 units/ml heparin. Samples were taken at 0, 2, 5, 7, and 10 min for assay of hepatic lipase activity. The perfusate samples were stabilized by the addition of Triton-N101 (0.01%) and glycerol (30%). At the end of heparin perfusion, the hepatic lipase-depleted liver was flushed for 10 min in single pass mode, followed by recycling perfusion with medium containing radiolabeled remnants for 15 min. The liver was then flushed and homogenized as described above.

Experiments with hepatic lipase antiserum

After a 30-min recovery period as described above and flushing for 10 min in a single pass mode, 60 ml of fresh perfusate containing goat anti-rat hepatic lipase (4–9 mg) or nonimmune serum (goat IgG, 4–9 mg) was recirculated for 15 min. ^{3}H-labeled remnants were then added to this perfusate and the livers were perfused for a further 15 min, during which samples (1.4 ml) were taken and livers were homogenized as described above.

Isolation of endosomes

Endosome fractions were separated from liver homogenates as described by Belcher et al. (14). Briefly "supernatant 3" containing impure endosomes was diluted with isotonic Percoll (Pharmacia, Pistcataway, NJ), pH 7.4, at a final density of 1.07 g/ml and centrifuged at 29,000 g_{av} for 45 min. The upper portion of the tube contents enriched in endosomes ("crude endosomes") was removed, diluted with 0.15 M NaCl, and then sedimented onto a sucrose "cushion" (2.5 M) to separate a purified endosome-rich fraction.

Assay of hepatic triglyceride lipase

Hepatic lipase activity was determined with a sonicated dispersion of [^{3}H]triolein emulsified with gum arabic (0.5%) as described by Ehnholm and Kuusi (15). The reaction mixture (0.5 ml, pH 8.4) contained 0.025 μM [^{3}H]triolein, 0.2 M Tris, 1.0 M NaCl, 10 mM $CaCl_2$, 1% bovine serum albumin. The reaction was initiated by the addition of a sample of perfusate or liver homogenate fraction (40 μl). Incubation was at 29°C for 30 min, after which the reaction was terminated by the addition of 3.25 ml methanol–chloroform–heptane 1.41:1.25:1.0 (v/v/v) and 0.5 ml K_2CO_3 (0.5 M, pH 10). The released [^{3}H]oleic acid in the aqueous phase was determined as described by Belfrage and Vaughn (16).

Analytical techniques

Lipids in perfusates and homogenate fractions were extracted into chloroform–methanol 1:1 (17) to which butylated hydroxytoluene (BHT) was added at a final concentration of 10 μg/ml. After separation of the two phases, samples of the chloroform layer were taken for assay of ^{3}H as described below. Extracted lipids were separated by thin-layer chromatography on Silica Gel G plates (Analtech, Newark, DE) that had been washed in acetone and activated for 35 min at 110°C. Plates were developed in hexane–diethyl ether–formic acid 80:20:2 (v/v/v) (18). Spots corresponding to the standards for cholesteryl esters (R_f 0.67–0.76), triglycerides (R_f 0.45–0.56), free fatty acids (R_f 0.30–0.37), diglycerides (R_f 0.07–0.17), and phospholipids + monoglycerides (origin) were identified after exposure to iodine vapor, scraped off into 5 ml Betamax (ICN-Biomedical Inc., Irvine, CA) and assayed for ^{3}H by liquid scintillation spectrometry. Quenching was determined with an internal standard. Recovery of ^{3}H lipids from the plates exceeded 90%.

Proteins in perfusate samples were determined with bicinchoninic acid (BCA reagent, Pierce, Rockford, IL) and in liver samples as described by Petersen (19). ApoE was measured by radioimmunoassay (20). Total triglycerides and cholesterol in chylomicrons and remnants were estimated by enzymatic techniques in a Roche Cobas Mira analyzer (21, 22).

Calculations and statistical analysis

The [^{3}H]palmitate infused into the duodenum labeled primarily chylomicron triglycerides and cholesteryl esters. During hydrolysis of chylomicron lipids by lipoprotein lipase, most of the triglycerides are hydrolyzed, but cholesteryl esters are not. Hence, the ratio of [^{3}H]palmitate in cholesteryl esters to that in triglycerides increases and can be used to calculate the percentage hydrolysis of chylomicron triglycerides during the formation of chylomicron remnants (23). After chylomicron remnants are taken up by the liver, their component triglycerides are rapidly hydrolyzed (see Results). In the liver, however, unesterified [^{3}H]palmitate released from the triglycerides is rapidly re-esterified to triglycerides and other lipid esters, mainly phospholipids (24). Therefore, the same ratio cannot be used to calculate the further hydrolysis of remnant triglycerides in extracts of whole liver. In endosomes, however, this ratio can be so used provided that: *1)* cholesteryl esters are not hydrolyzed on the cell surface or in endosomes; and *2)* endosomal triglycerides do not contain any re-esterified [^{3}H]palmitate. The first assumption is supported by data showing that cholesteryl esters of endocytosed LDL are not hydrolyzed in hepatocytic endosomes (25). The second assumption is reasonable because the enzymes required for triglyceride synthesis are confined to the endoplasmic reticulum and mitochondria of hepatocytes (26). Use of the ratio to calculate triglyceride hydrolysis in remnants within endosomes also requires that isolated endosomal fractions be virtually free of contamination by organelles that contain re-esterified triglycerides, including endoplasmic reticulum, Golgi apparatus, and lipid droplets.

All primary data were taken as the average of duplicate assays for individual experiments and the final results are expressed as mean ± SD. Significance of differences between the experimental groups was evaluated by Student's paired *t*-test.

RESULTS

Effect of heparin and hepatic lipase antiserum on hepatic lipase activity and apoE in liver perfusates and homogenates

The effects of heparin and rat hepatic lipase antiserum on hepatic lipase activity were estimated in perfused livers. For heparin experiments, perfusate containing heparin (25 units/ml) was recycled for 10 min before the addition of radiolabeled remnants as described in Methods. Lipase activity was measured in the liver and perfusates containing heparin. **Fig. 1** shows total hepatic lipase activity in the perfusates and homogenates of control and treated livers. Heparin released 74.4 ± 11.4% of hepatic lipase activity whereas only 8.65 ± 4.62% was found in control perfusates. The residual activity in heparin-preperfused livers fell to 23.4% of that of control livers. Release into perfusates was maximal after 7 min of perfusion with heparin and then remained constant. Hepatic lipase activity in perfusate washes after 15 min of perfusion with chylomicron remnants as described below was negligible (heparin washes = 0.48 ± 0.15 μmol • min^{-1}; control washes = 0.16 ± 0.041 μmol • min^{-1}). Thus, most of the hepatic lipase activity on liver cell surfaces was displaced by heparin, so that the amount released in the subsequent flushes did not exceed 2% of the amount originally displaced.

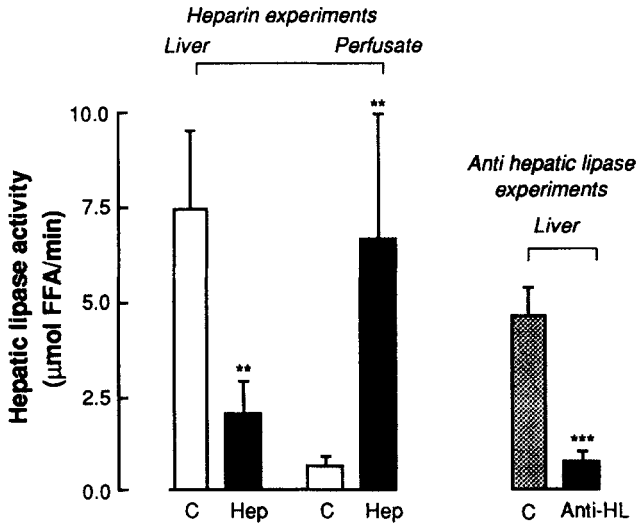

Fig. 1. Hepatic lipase activity in liver perfusates and liver homogenates. For experiments with heparin, livers were perfused for 10 min with perfusate containing heparin (25 units/ml) or perfusate alone. After subsequent perfusion for 15 min with chylomicron remnants, livers were homogenized. Hepatic lipase activity was assayed in perfusates and liver homogenates. In experiments with anti-rat hepatic lipase, perfusate containing affinity-purified antiserum or nonimmune serum was recycled for 15 min. Chylomicron remnants were then added and perfusions continued for an additional 15 min. C, control (open columns); Hep, heparin (dark stippled column); antibody control (C, light stippled column) and anti-hepatic lipase (Anti-HL, black column). Values are mean ± SD for four paired rat livers. Differences from controls: $^{**}P < 0.03$; $^{***}P < 0.003$.

Heparin also released apoE into the perfusates. The amount of apoE released into the perfusate by heparin during 10 min, measured in one experiment, was about twice that of the control liver (29.1 μg and 14.8 μg, respectively). To evaluate the release of apoE further, perfused livers were pulsed with heparin or suramin. After recirculating perfusion for 30 min, single pass perfusion was instituted with 10% erythrocytes. After 5 min, suramin or heparin was injected into the system just proximal to the portal vein. Results of an experiment in which two boluses of suramin were injected are shown in **Fig. 2.** Release of apoE was virtually instantaneous, with a peak 20 sec after injection of 10 nmol suramin. Release was smaller after the second bolus. In other experiments, heparin in doses up to 250 units was considerably less effective, releasing 10–20% of the mass released by suramin (not shown). In these experiments, relatively little triglyceride was released with the apoE (Fig. 2), and much smaller masses of apoA-I were rapidly released (about 2% of that of apoE).

For experiments with hepatic lipase antiserum, perfusate containing either nonimmune goat-IgG or affinity-purified IgG prepared from goat hepatic lipase antiserum was allowed to recirculate for 15 min, after which remnants were added to the same perfusate and recycling continued for a further 15 min. Hepatic lipase activity in the liver homogenates was reduced to 16.8 ± 17.0% of the activity found in control homogenates (Fig. 1). Similar results were also obtained with endosome-rich fractions isolated from the antibody-treated and control livers (0.026 ± 0.02 (n = 4) versus 0.16 ± 0.11 $\mu mol \cdot min^{-1} \cdot ml^{-1}$ (n = 4), i.e., reduction to 18.8 ± 10.5%). These results show that both heparin and rat hepatic lipase antiserum reduced the hepatic lipase activity in liver but that the antiserum was somewhat more effective.

Effect of heparin and hepatic lipase antiserum on the uptake of ^{3}H-labeled triglycerides and cholesteryl esters of chylomicron remnants by the perfused liver

Fig. 3 shows data on the removal of ^{3}H-labeled triglycerides and cholesteryl esters of chylomicron remnants during 15 min of recycling perfusion. When control or heparin-preperfused isolated livers were perfused by recirculation of radiolabeled chylomicron remnants (1 mg triglycerides/0.22–0.38 mg total cholesterol), ^{3}H-labeled triglycerides (Fig. 3-A, C) and cholesteryl esters (Fig. 3-B, D) were cleared from the perfusates at similar rates. Five minutes after the addition of radiolabeled remnants, 45.0% of the radiolabeled triglycerides and 46.8% of the cholesteryl esters were cleared from the perfusates of control livers, values significantly greater than the 30.5% of the triglycerides and 36.9% of the cholesteryl esters cleared by the heparin-preperfused livers. After 10 min, 46.3% of ^{3}H-labeled triglycerides had been cleared from perfusates of heparin-preperfused livers compared with 71.7% in the controls.

Hepatic lipase antiserum reduced the clearance of [^{3}H]triglycerides and cholesteryl esters substantially (Fig. 3). The reduction in clearance was highly significant: after 10 min clearance of [^{3}H]CE and [^{3}H]TG was 28.3%

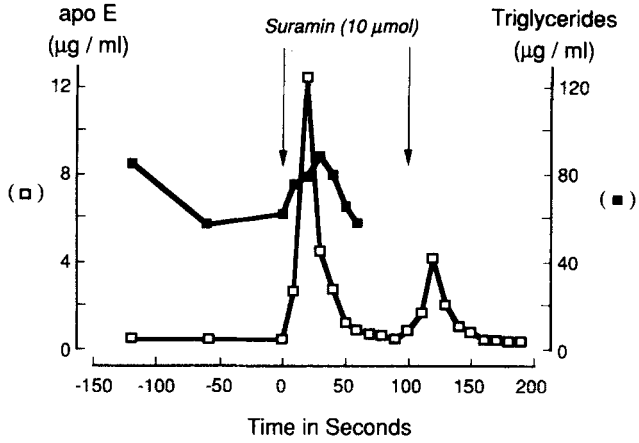

Fig. 2. Injection of suramin releases apoE from perfused rat liver. After recirculating perfusion for 30 min, as described in Methods, single pass perfusion was begun. After 2 min, suramin was injected into the portal venous catheter and 10-sec samples of effluent perfusate samples were collected. As shown, a second injection of suramin released less apoE into the perfusate than the first injection. A small amount of triglycerides was also released (in mass about double the release of apoE).

and 26.3%, respectively, as compared with 50.6% and 54.6% in controls. This inhibition by the antiserum was greater than that observed after depletion of hepatic lipase with heparin.

At the end of the perfusions the livers were flushed for 5 min to wash out the residual perfusate, and total ^3H taken up by the livers was determined after homogenization and extraction of lipids. As shown in **Fig. 4,** 68.7% of the total lipids added to the perfusate was recovered in the control livers as compared to the 44.3% in the heparin-preperfused livers ($P < 0.05$). In antiserum-treated livers, only 24.8% of the labeled lipids was recovered ($P < 0.005$). The total recovery of ^3H in the perfusates and liver homogenate after 15 min was almost complete (94.9 ± 0.79% for control vs. 94.7 ± 0.76% for heparin-preperfused livers; 95.8 ± 2.17% for control vs. 94.6 ± 2.74% for antiserum-treated livers). Recovery of cholesteryl esters in the control livers was greater than that of triglycerides (104.8 ± 11.1% compared to 83.7 ± 10.7%), suggesting that some triglycerides in the livers had been hydrolyzed.

Hydrolysis of triglycerides of chylomicron remnants by hepatic lipase

To gain a more precise measure of the extent of hydrolysis of triglycerides of chylomicron remnants taken up by the liver, we examined the distribution of ^3H in lipids separated by thin-layer chromatography of chylomicrons,

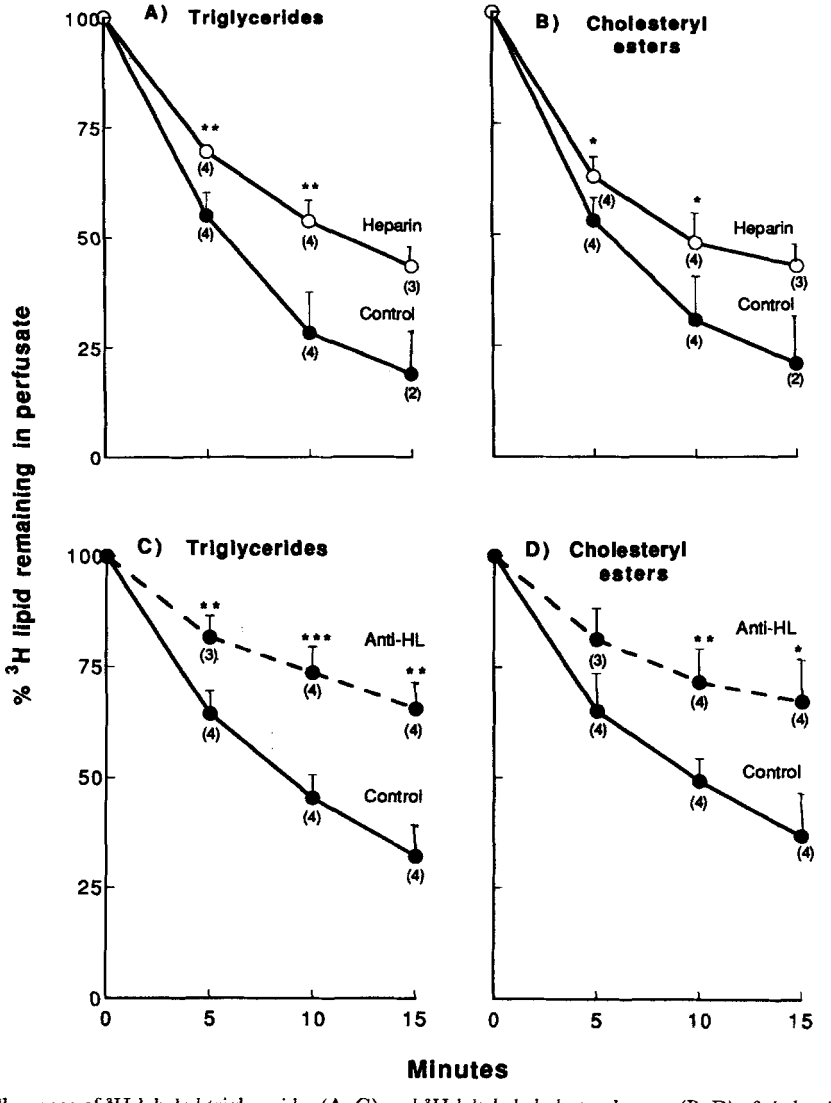

Fig. 3. Clearance of ^3H-labeled triglycerides (A, C) and ^3H-labeled cholesteryl esters (B, D) of chylomicron remnants from recirculating perfusates of control and heparin-preperfused (Heparin); and antibody control and anti-rat hepatic lipase perfused (Anti-HL) isolated rat livers. Values are mean ± SD and expressed as percent of added ^3H lipids remaining in perfusates. Differences between means for control and treated livers: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Number of determinations (n).

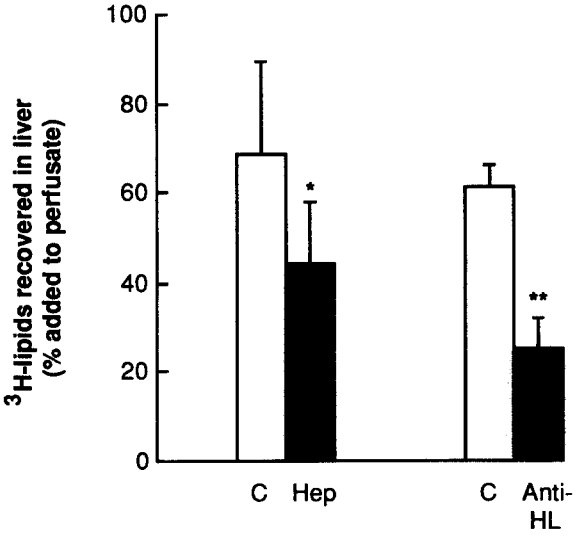

Fig. 4. Recovery of ^{3}H-labeled lipids in control (C, open column) and heparin-preperfused (Hep, stippled column) livers and in antibody control (c, nonimmune serum) and anti-hepatic lipase (Anti-HL, black column) perfused livers 15 min after the addition of radiolabeled remnants to recirculating perfusates. Values are mean ± SD from four rat livers. Differences between mean values for control and treated livers: $^*P < 0.05$; $^{**}P < 0.005$.

chylomicron remnants, and liver fractions (**Fig. 5** and **Fig. 6**). The ^{3}H was distributed mainly in the triglycerides, cholesteryl esters, and phospholipids, with small amounts in free fatty acids (FFA) and diglycerides. In chylomicrons, 86–90% of the tracer was in triglycerides and less than 7% in cholesteryl esters and phospholipids. In chylomicron remnants, as expected, more of the ^{3}H was in cholesteryl esters. From the [^{3}H]CE:[^{3}H]TG ratios of chylomicrons and chylomicron remnants, it was calculated that 75–86% of the triglycerides were removed during remnant formation in the functionally eviscerated rats. The distribution of perfusate lipids closely resembled that of the chylomicron remnants, indicating little hydrolysis by hepatic lipase (Figs. 5 and 6). In control liver homogenates, further reduction of ^{3}H in triglycerides was accompanied by a substantial increase of ^{3}H in phospholipids, indicating that triglycerides had been hydrolyzed and re-esterified. Lesser increases in ^{3}H-labeled phospholipids were observed in endosome fractions, reflecting re-esterification in other compartments of the cell and subsequent exchange/transfer to endosome membranes.

These changes related to triglyceride hydrolysis were significantly reduced in heparin-preperfused liver homogenates and crude endosomes ($0.05 > P > 0.005$) (Fig. 5). No FFA accumulated in perfusates of either control or heparin preperfused livers (< 2% of total ^{3}H). In control and heparin preperfused liver homogenates and their endosomes, approximately 6–7% and 4–5% of ^{3}H, respectively, was in FFA.

Major differences were observed in the distribution of lipids in triglycerides and phospholipids of liver homogenates and endosome fractions from livers treated with hepatic lipase antiserum as compared to their controls (Fig. 6). These differences, which were highly significant, suggested a marked inhibition in hydrolysis of triglycerides by the antiserum. For endosome fractions, the extent to which triglyceride hydrolysis was inhibited was estimated from changes in the ratio of [^{3}H]CE:[^{3}H]TG (see Methods). Triglycerides of remnants in endosomes were extensively hydrolyzed in control livers (**Table 1**). Preperfusion with heparin reduced the extent of hydrolysis moderately whereas hydrolysis was reduced by about 91% by hepatic lipase antiserum.

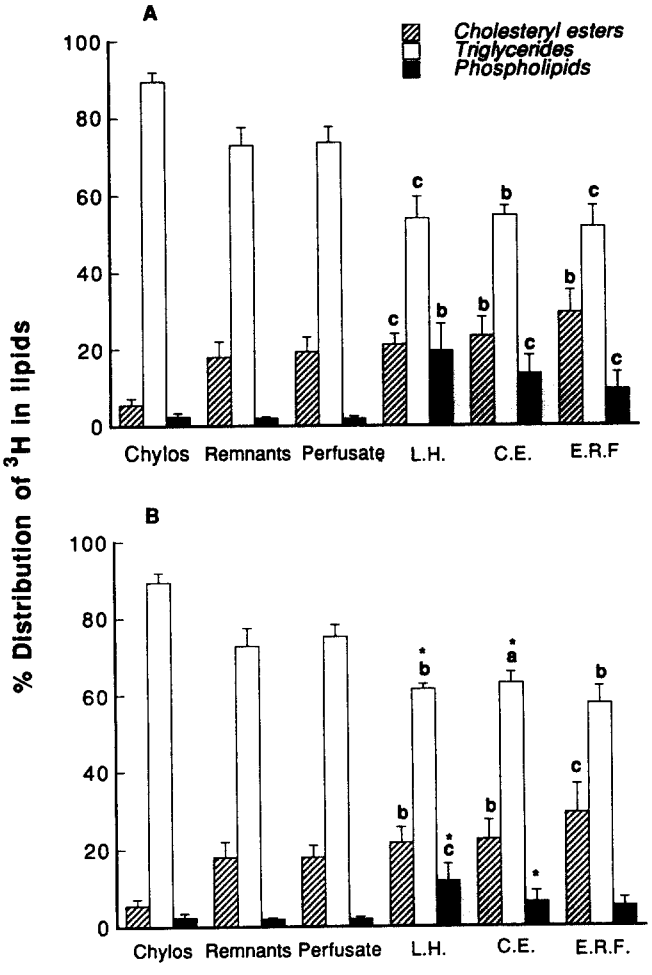

Fig. 5. Percentage distribution of ^{3}H-labeled cholesteryl esters, triglycerides and phospholipids in chylomicrons (Chylos), remnants, final perfusate (15 min), liver homogenates (L.H.), crude endosomes (C.E.), and endosome-rich fractions (E.R.F.), in control (A) and heparin-preperfused livers (B). Values are mean ± SD, n = 4 except for E.R.F. in control livers (n = 3). Differences from cholesteryl esters, triglycerides, and phospholipids of remnants: $^aP < 0.005$; $^bP < 0.01$; $^cP < 0.05$. Differences between control and heparin-preperfused livers; $^*P < 0.05$.

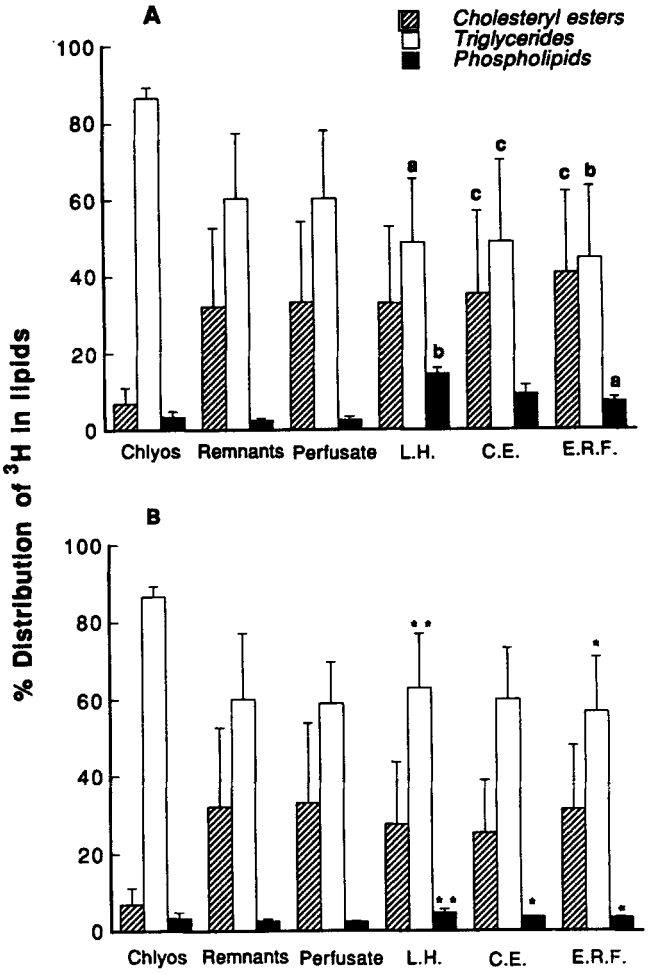

Fig. 6. Percentage distribution of ^{3}H-labeled cholesteryl esters, triglycerides, and phospholipids in chylomicrons (Chylos), remnants, final perfusate (15 min), liver homogenates (L.H.), crude endosomes (C.E.), and endosome-rich fractions (E.R.F.) in control (A) and anti hepatic lipase-treated livers (B). Values are mean ± SD from three rat livers. Differences from cholesteryl esters, triglycerides, and phospholipids of remnants: $^aP < 0.005$; $^bP < 0.01$; $^cP < 0.05$. Differences between control and anti-hepatic lipase-treated livers: $^*P < 0.05$; $^{**}P < 0.01$.

As described in Methods, it was not possible to calculate percent hydrolysis of triglycerides in the liver homogenates, because re-esterification of fatty acids to form triglycerides presumably occurred together with re-esterification to form phospholipids. This would yield an underestimate of hydrolysis, although a minimum estimate could be calculated from the increase in ^{3}H in phospholipids. Endosomes, on the other hand, would not be expected to contain re-esterified triglycerides, which are synthesized in the endoplasmic reticulum and transferred to fat droplets.

Endocytosis of remnant lipids

The extent to which remnant lipids were endocytosed was estimated from the recovery of [^{3}H]CE and [^{3}H]TG in the partially purified endosome-rich fractions (**Table 2**). As expected, both labeled lipids were taken up into the endosomes. In each paired comparison, a larger fraction of each labeled lipid present in the liver homogenates was recovered from livers in which hepatic lipase activity had been reduced. This difference was significant for both CE and TG in the experiments with hepatic lipase antiserum and for all data combined. These results suggest that the endocytosis of remnants taken up by the liver was facilitated when hepatic lipase was inhibited.

Effect of heparin and hepatic lipase antiserum on the overall recovery of ^{3}H-labeled lipids in liver

Table 3 shows the percent of added ^{3}H-labeled triglycerides, cholesteryl esters, and phospholipids found in perfusates, liver homogenates, and endosome fractions 15 min after recirculation of radiolabeled chylomicron remnants through control and treated livers. Uptake of [^{3}H]cholesteryl esters serves as a marker of particle uptake and endocytosis. Uptake was reduced by 33% in liver homogenates of heparin-treated livers as compared to their controls, and by 65% in homogenates of antiserum-treated livers ($0.01 > P > 0.001$ in both cases). Greater reduction of [^{3}H]triglyceride uptake was also observed in the antiserum-treated as compared to heparin-preperfused livers, and this was reflected by a more substantial decrease of ^{3}H in phospholipids in liver homogenates of antiserum-treated livers as compared to the heparin-preperfused livers. The amount of ^{3}H in endosome-cholesteryl esters was reduced to a much lesser

TABLE 1. Hydrolysis of ^{3}H-labeled triglycerides of chylomicron remnants (calculated from CE:TG ratios) in perfusates and endosome fractions after 15 min of recycling perfusion

	Percent [^{3}H]Triglyceride Hydrolyzed			
Fraction	Control	Heparin	Control	Anti-HL
Final perfusates	5.09 ± 5.89	1.75 ± 3.49	2.55 ± 1.84	1.08 ± 2.16
Crude endosomes	41.9 ± 7.10	30.4 ± 2.76^a	37.6 ± 14.9	3.81 ± 7.64^a
Endosome-rich fractions	60.7 ± 10.24	50.3 ± 10.1^b	54.1 ± 12.31	4.14 ± 4.78^b

Values are means ± SD from four rat livers in each group.
Differences from control: $^a0.05 > P > 0.01$; $^bP < 0.01$.

TABLE 2. Effect of heparin or anti-rat hepatic lipase on recovery of ^{3}H-labeled cholesteryl esters and triglycerides in endosome-rich fractions isolated 15 min after perfusions containing radiolabeled chylomicron remnants

	Percent of Liver Homogenate	
Liver	[^{3}H]Cholesteryl Esters	[^{3}H]Triglycerides
Control	7.31 ± 3.49	4.99 ± 2.04
Heparin-preperfused (n = 4)	10.2 ± 4.25	6.94 ± 2.74
Control	5.68 ± 2.26	4.32 ± 2.45
Anti-hepatic lipase (n = 4)	8.27 ± 2.33[a]	6.48 ± 2.07[b]
All control (n = 8)	6.50 ± 2.86	4.66 ± 2.11
All treated (n = 8)	9.23 ± 3.33[b]	6.71 ± 2.26[b]

Values are means ± SD; number of experiments (n).
Differences between control and anti-hepatic lipase-treated livers: [a]$P < 0.02$; [b]$P < 0.01$.

extent in each case, consistent with a greater efficiency of endocytosis when hepatic lipase was inhibited.

DISCUSSION

The principal findings of this study are that depletion of hepatic lipase or its inhibition *1)* reduces the rate of uptake of chylomicron remnants by the isolated, perfused rat liver and *2)* augments the endocytosis of those chylomicron remnants that are taken up by the liver under these conditions. The first observation is consistent with the demonstration by Daggy and Bensadoun (4) that injection of hepatic lipase antiserum led to the accumulation of triglyceride-rich lipoproteins in blood plasma of fat-loaded rats, and specifically caused apoB-48 to accumulate in intermediate density lipoproteins and LDL. This observation also confirms the report by Sultan and associates (6) that the removal of cholesteryl linoleyl ether-labeled chylomicron remnants from the blood and uptake by the liver during a 5-min period in vivo was reduced about 50% by preinjection of goat antiserum against rat hepatic lipase. As in our work, the latter investigators found that injection of antiserum in vivo inhibited about 85% of total hepatic lipase in liver homogenates. Whereas they prepared their remnants from large lymph chylomicrons of fat-fed rats, we used small chylomicrons from glucose-fed rats. It thus appears that our results are not related to use of an unusually small population of remnant particles. Hamilton (27) initially observed that hepatic lipase fails to act upon triglycerides in large thoracic duct chylomicrons, but would hydrolyze these triglycerides when acting in concert with other lipases present in post-heparin plasma in vivo (i.e., lipoprotein lipase). There is ample evidence that lipoprotein lipase is required for hydrolysis of large chylomicrons, whereas hepatic lipase is at least equally active against triglycerides in smaller lipoproteins, including those of LDL (28,

TABLE 3. Percent of added ^{3}H-labeled triglycerides, cholesteryl esters, and phospholipids recovered in perfusate, liver homogenate, crude endosomes, and endosome-rich fractions 15 min after recirculation of radiolabeled chylomicron remnants through control, heparin-preperfused (Heparin), and anti-hepatic lipase IgG (Anti-HL)-perfused rat livers

	Percent of ^{3}H Added to Perfusate					
	Cholesteryl Esters		Triglycerides		Phospholipids	
Fraction	Control	Heparin	Control	Heparin	Control	Heparin
Perfusate (15 min)	26.4 ± 11.7	40.6 ± 6.34[a]	26.3 ± 12.3	42.2 ± 6.17[a]	23.8 ± 14.0	42.0 ± 5.77
Liver homogenate	78.6 ± 19.9	52.9 ± 15.2[b]	52.2 ± 18.9	37.7 ± 12.4	587.1 ± 110.1	268.4 ± 142.9[a]
Crude endosomes	11.0 ± 3.49	9.80 ± 2.81	6.26 ± 2.72	6.48 ± 2.81	65.5 ± 17.9	23.9 ± 10.9
Endosome-rich fraction	5.43 ± 4.03*	5.69 ± 3.21*	2.66 ± 2.39*	2.61 ± 1.21*	14.8 ± 8.97*	8.10 ± 5.80*
	Control	Anti-HL	Control	Anti-HL	Control	Anti-HL
Perfusate (15 min)	39.2 ± 2.17	68.1 ± 9.05[b]	39.3 ± 6.16	65.6 ± 5.52[c]	48.6 ± 5.58	74.4 ± 6.45*[a]
Liver homogenate	65.5 ± 12.7	23.0 ± 7.42[b]	49.7 ± 6.97	24.8 ± 6.93[a]	388.3 ± 161.2	57.1 ± 23.3[a]
Crude endosomes	11.1 ± 3.79	5.34 ± 2.32	8.08 ± 3.96	6.12 ± 1.34	36.1 ± 11.5	13.2 ± 2.27[a]
Endosome-rich fraction	3.75 ± 1.67	1.88 ± 0.74	2.24 ± 1.54	1.56 ± 0.26	7.32 ± 1.16	3.62 ± 1.32[a]

Values are means ± SD; n = 4 except * (n = 3).
Significance of differences from control: [a]$0.05 > P > 0.01$; [b]$0.01 > P > 0.001$; [c] < 0.001.

29). Griglio, Sultan, and LaGrange (30) observed that, in vitro, triglycerides of large chylomicron remnants were a poor substrate for hepatic lipase, whereas phosphatidylcholine of the remnants was readily hydrolyzed to lysophosphatidylcholine. These investigators also showed that remnants that had been hydrolyzed by hepatic lipase were more readily bound to hepatic plasma membranes and isolated rat hepatocytes and were taken up more rapidly from the circulation by the liver in vivo (7, 31). Their results are thus consistent with those of Borensztajn, Getz, and Kotlar (32) who reported that hydrolysis of chylomicron phospholipids increased the rate of chylomicron uptake by the rat liver.

We used two methods to deplete hepatic lipase activity in the liver. Both yielded similar results, although inhibition of lipase activity with specific antibodies was more effective with respect to uptake by the perfused rat liver than was removal of hepatic lipase from accessible sites in the liver by heparin. When we initiated this work, we were aware that apoE is present on the microvillous (basolateral) surface of hepatocytes (33) and we found that heparin not only displaces hepatic lipase but apoE as well. Similar observations with respect to displacement of apoE by heparin have recently been reported for HepG2 cells (34). Displacement of apoE, like that of hepatic lipase (35), was very rapid, indicating the presence of a readily accessible pool. This apoE may be bound, like hepatic lipase, to glycosaminoglycans, such as heparan sulfate, which is known to be associated with the sinusoidal surface of hepatocytes (36). As we failed to observe appreciable release of triglycerides with apoE when heparin or suramin was pulsed through isolated livers, we conclude that this pool of apoE is probably not associated with triglyceride-rich lipoproteins on the surface, consistent with immunocytochemical data (33).

During recycling perfusion with heparin, the amount of apoE released into the perfusate was small and, with single pulses, heparin appeared less effective than suramin in releasing apoE. It thus seems unlikely that depletion of apoE from accessible sites in the liver contributed to the reduced uptake of chylomicron remnants in the experiments in which hepatic lipase was depleted by preperfusion with heparin. Although that possibility cannot be excluded, it is notable that heparin was less effective than lipase antiserum in reducing remnant uptake.

Even with almost complete inhibition of hepatic lipase activity by antiserum, remnant uptake into the perfused liver continued at an appreciable rate, as it did in the in vivo studies of Sultan et al. (6), suggesting that alternative mechanisms account for some of the observed uptake. In this regard, it is notable that the extent of endocytosis of remnants taken up by the liver during the 15 min of perfusion was actually increased when hepatic lipase activity was reduced. As in the estradiol-treated rat, chylomicron remnants might initially bind mainly to endocytic receptors when hepatic lipase was inhibited in the current experiments. With this scenario, then, there may be two mechanisms for the initial uptake of chylomicron remnants by the perfused liver and by the liver in vivo: one involves hepatic lipase-dependent binding, and the other binding to endocytic receptors, such as the LDL receptor or the LRP. Binding to the first site would not lead directly to endocytosis, but would require further processing before the remnant particle binds to an endocytic receptor. By contrast, initial binding to an endocytic receptor would be followed by more rapid endocytosis. In the estradiol-treated rat, hepatic lipase activity is reduced (37), which also may promote initial binding to the LDL receptor.

Ishihara, Fedarko, and Conrad (38) have shown that the major heparan sulfate proteoglycan in a rat liver cell line is a glycosyl phosphatidyl inositol-anchored protein. Such proteins may be associated with caveolae, which are membrane pits that lack a clathrin coat (39). Whether caveolae actually pinch off and internalize receptor-bound ligands localized to this site is uncertain (39) but, if this does occur, chylomicron remnants bound to hepatic lipase could thereby be internalized, perhaps at a slower rate than occurs via coated pits.

ApoE, like hepatic lipase, may be bound to glycosaminoglycans on the surface of hepatocytes. ApoE on the surface of remnants could also bind to glycosaminoglycans, such as heparan sulfate, as suggested by experiments of Oswald and associates (40) with rat hepatocyte monolayers and by Ji and associates (41) with human hepatoma cells and fibroblasts, and Chinese hamster ovary cells. Recently, Eisenberg and associates (42) have shown that lipoprotein lipase-enhanced binding of lipoproteins to HepG2 cells and fibroblasts is mediated by heparan sulfate. Remnants containing lipoprotein lipase could then associate with more apoE as well as hepatic lipase. However, the reduced uptake of remnants in livers pretreated with hepatic lipase antiserum suggests that this enzyme itself may have a major role as a binding site for remnant particles. The association of chylomicron remnants with hepatic lipase could also promote remnant binding to endocytic receptors, as proposed for lipoprotein lipase by Beisiegel, Weber, and Bengtsson-Olivecrona (43) with respect to LRP. Chappell and associates (44) have recently shown that lipoprotein lipase bound to LRP undergoes endocytosis and the same could apply to hepatic lipase. Relevant to this observation is the finding by Belcher and associates (45) that hepatic lipase is highly concentrated in endosomes of rat hepatocytes.

As observed by Griglio and associates (30), hydrolysis of phospholipids by hepatic lipase may promote binding to endocytic receptors. Similarly, Evans and associates (46) have found that uptake of hypertriglyceridemic human VLDL into HepG2 cells is promoted by lipolysis. Brasaemle and associates (8) have observed that hydroly-

sis of remnant lipids by hepatic lipase greatly increases the exposure of apoE to specific antibodies; such a conformational change could facilitate binding to endocytic receptors. Alternatively, the hydrolysis of surface lipids by hepatic lipase may facilitate binding of apoE on liver cell surfaces to remnant particles. Mixing apoE with rabbit β-VLDL increases the affinity of the lipoprotein particles to LRP (47).

In our experiments, triglycerides of chylomicron remnants were extensively hydrolyzed as they were taken up from the perfusates, and this hydrolysis was partially inhibited by preperfusion with heparin and almost completely inhibited by treatment of the livers with lipase antiserum. Because hepatic lipase is present not only on surface sites in the liver, but also in hepatocytic endosomes (45), it is not immediately evident whether lipolysis occurs extracellularly, within endosomes, or both. The appreciable hydrolysis after preperfusion with heparin raises the possibility that hydrolysis continued within endosomes, which initially would not be depleted by heparin-perfusion of the livers. Secretion of newly synthesized hepatic lipase or recycling of endosomal hepatic lipase to the cell surface after heparin perfusion could also account for the incomplete effect of this method of lipase depletion. In the case of treatment with antiserum, hepatic lipase activity within endosomes was also depleted. There is, however, no reason to suppose that hepatic lipase would not act upon remnant phosphatidylcholine as well as triglycerides prior to endocytosis and it seems likely that both extracellular and intracellular hydrolysis occurred during the 15-min period of perfusion. In any event, the fatty acids released were rapidly re-esterified, in part to phospholipid precursors. The released [^{3}H]palmitate was almost certainly re-esterified to triglycerides in the endoplasmic reticulum and the greater than complete recovery of ^{3}H in cholesteryl esters in the liver and final perfusate suggests that a small amount may have been re-esterified with cholesterol. These events presumably do not occur within endosomes, so that the recovery of radioactivity in endosomal cholesteryl esters remains a valid marker of the endocytic process in experiments in vivo as well as in perfused livers, provided that the endosomes are uncontaminated by other organelles or by fat droplets. Both of these have been virtually excluded by our previous studies of endosome fractions (2, 14).

Hepatic lipase has long been considered to reside almost exclusively on the surface of endothelial cells in the liver, based mainly upon the immunocytochemical studies of Kuusi and associates (48). This interpretation has, however, been challenged by Marteau and associates (49) and is also called into question by the demonstration by Belcher and associates (45) that hepatic lipase is highly concentrated in hepatocytic endosomes, an observation subsequently confirmed (50). Although further data are required to ascertain the distribution of hepatic lipase on or near the surface of liver cells, available evidence suggests that at least some of the enzyme is bound to heparan sulfate on hepatocytes and is thus in close proximity to endocytic receptors.

The results of the current research together with earlier observations suggest that hepatic lipase facilities, but is not essential for, the initial uptake of chylomicron remnants by the rat liver. Furthermore, hepatic lipase activity is not required for the internalization of remnant particles leading to lysosomal catabolism. The extent to which chylomicron remnants bind initially to hepatic lipase or endocytic receptors will vary with their respective surface densities and may also be a function of the remnant load. Not only may the affinities of the binding proteins differ, but their rates of unloading and regeneration (for example, by receptor-recycling) could vary with load. We predict, however, that the phenotype of hepatic lipase deficiency does not include massive accumulation of chylomicron remnants in the blood. This prediction is consistent with observations in cats (a species with low hepatic lipase activity) by Demacker, Hijmans, and Stalenhoef (5) who observed gradual accumulation of hepatogenous VLDL but not of particles containing apoB-48 during prolonged inhibition of hepatic lipase by specific antiserum in vivo, and by studies in humans with hepatic lipase deficiency (51).

Our current results, as well as our earlier work (2, 3), do make it clear that measurement of the rate of initial uptake of triglyceride-rich lipoproteins by liver is not sufficient to evaluate the overall processes by which their components are processed and metabolized. Huettinger and associates (52) have shown that lactoferrin inhibits endocytosis of chylomicron remnants by rat liver in vivo to a greater extent than total uptake from the blood by the liver. We have made similar observations with the 39 kDa LRP-associated protein (H. Mokuno, J. Herz, S. Brady, and R. J. Havel, unpublished data). Although the precise mechanisms by which the initial uptake and endocytosis of remnant particles are coupled remain to be elucidated, hepatic lipase appears to have an important role in chylomicron remnant uptake and processing in the rat.

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