Chylomicron-Chylomicron Remnant Clearance by Liver and Bone Marrow in Rabbits

FACTORS THAT MODIFY TISSUE-SPECIFIC UPTAKE*

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M. Mahmood Hussain, Robert W. Mahley†, Janet K. Boyles, Menahem Fainaru, Walter J. Brecht, and Peter A. Lindquist

From the Gladstone Foundation Laboratories for Cardiovascular Disease, Cardiovascular Research Institute, Departments of Pathology and Medicine, University of California, San Francisco, California 94140-0608

The metabolism of [14C]cholesterol- and [3H]retinol-labeled chylomicrons obtained from canine thoracic duct or rabbit mesenteric lymph was investigated in normal fasted rabbits. Typically, 70–80% of the chylomicrons injected into the rabbits were cleared from the plasma in 20 min, and their uptake was accounted for principally by the liver and the bone marrow. Surprisingly, the bone marrow was a major site of uptake; the uptake ranged from about half that of the liver to a nearly equal amount. The importance and specificity of chylomicron-chylomicron remnant uptake by the bone marrow were established by demonstrating that (a) bone marrow throughout the body accumulated these lipoproteins, (b) the level of uptake was consistent regardless of how the values were calculated or how the chylomicrons were prepared, (c) the uptake represented specific binding, and (d) radiolabeled intestinal lipoproteins induced in vivo delivered cholesterol and retinol to the marrow. Electron microscopic examination of the rabbit bone marrow established that perisinusoidal macrophages uniquely accounted for the uptake of the chylomicrons. Whereas liver cleared a variety of both triglyceride-rich lipoproteins (chylomicrons, chylomicron remnants, and very low density lipoproteins) and cholesterol-rich lipoproteins (β-very low density lipoproteins and high density lipoproteins containing apolipoprotein E), bone marrow uptake appeared to be restricted to the triglyceride-rich lipoproteins. More chylomicron remnants (generated in a hepatectomized rabbit) were cleared by the liver than by the bone marrow, and the addition of excess apolipoprotein E to chylomicrons resulted in their preferential uptake by the liver. The role of chylomicron-chylomicron remnant delivery of lipids or lipid-soluble vitamins to rabbit bone marrow is open to speculation, and whether triglyceride-rich lipoprotein uptake occurs to a significant extent in the bone marrow of humans remains to be determined.

Chylomicrons, originally designated by Gage and Fish (1) as large particles induced by the dietary consumption of lipids, enter the plasma through the mesenteric and thoracic duct lymph. Thereafter, triglyceride hydrolysis occurs rapidly via the action of endothelial cell-bound lipoprotein lipase (2–5). This process results in the formation of chylomicron remnants (6, 7), which are cleared from the plasma primarily by the liver (8, 9). Studies involving liver perfusion, cell cultures, and membrane binding have been designed to understand the molecular basis of chylomicron remnant removal by the liver (10–23). These studies have demonstrated that chylomicron remnant clearance by the liver is mediated by apolipoprotein (apo) E (15–18, 20) and may be modulated by apoC and phospholipids (16, 18, 23). Although the precise mechanism responsible for hepatic uptake of the chylomicron remnants is poorly understood, the presence of a unique apoE or chylomicron remnant receptor has been postulated (for review, see Refs. 24 and 25).

The present study was undertaken in an attempt to define more precisely the mechanism of cellular uptake of chylomicrons and chylomicron remnants in the rabbit. The rabbit was chosen because of the extensive use of this model by Zilversmit and associates (26, 27) and Redgrave and associates (6, 28). In addition, the rabbit was selected because of the availability of the Watanabe heritable hyperlipidemic rabbit as a model in which to study chylomicron metabolism in an animal displaying defective low density lipoprotein receptors (29, 30). Our initial experiments in normal rabbits demonstrated that the liver accounted for only one-half to two-thirds of the radiolabeled chylomicrons cleared from the plasma. These data suggested that there was another major site of catabolism of plasma chylomicrons and chylomicron remnants in the rabbit. In this study we found that the other major site is the bone marrow and that both the liver and the bone marrow are important for the clearance of triglyceride-rich lipoproteins.

MATERIALS AND METHODS

Animals and Diets

Adult mongrel dogs (University of California, San Francisco) maintained under controlled laboratory conditions for more than 4 weeks were used for lymph collection. They were fed a control dog chow (Purina dog meal) ad libitum prior to their use. Cholesterol-fed foxhounds (Brink Farm, Paola, KS) were fed a coconut oil-cholesterol diet (Teklad Mills, Madison, WI) as described previously (31) and were the source of canine β-very low density lipoproteins (β-VLDL) and apoE-HDL. They were fed their diet ad libitum and had plasma cholesterol levels in excess of 750 mg/dl. Plasma for lipoprotein preparation was obtained after the animals were fasted overnight. Male New Zealand White rabbits (typically 2.5–3.5 kg), obtained from Animal West (Soquel, CA), were maintained on a Purina Rabbit Diet.

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† To whom correspondence should be addressed: Gladstone Foundation Laboratories, P. O. Box 40068, San Francisco, CA 94140-8008.

1 The abbreviations used are: apo, apolipoprotein; apoE-HDL, high density lipoproteins containing apolipoprotein E; VLDL, very low density lipoproteins.
The lipid composition of canine lipoproteins is shown in Table I.

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>% composition</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Triglycerides</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>92.6 ± 1.3</td>
</tr>
<tr>
<td>14C-Labeled chylomicrons (column-purified)</td>
<td>92.7 ± 3.3</td>
</tr>
<tr>
<td>Chylomicron remnants</td>
<td>84.5, 86.8</td>
</tr>
</tbody>
</table>

a Values obtained for chylomicrons from seven different preparations. The particles were typically 1000-5000 Å in diameter, as determined by negative-staining electron microscopy. The triglyceride/cholesterol ratio was typically 60-75:1.

b Values obtained for two preparations of chylomicron remnants prepared 30 min after injection into hepatectomized rabbits. The triglyceride/cholesterol ratio in the remnants was approximately 26:1 after 30 min in the circulation; however, this ratio decreased to approximately 10:1 after 120 min in the circulation.

Chow diet ad libitum. The rabbits were fasted overnight before in vivo metabolism studies.

Lipoprotein Isolation

Canine and Rabbit Chylomicron Preparations—The procedure for creating a lymph fistula was a modification of the method described by Rajpal and Kirkpatrick (32), as described earlier by Melchior et al. (33). Briefly, the animals were anesthetized with halothane and the veins in the vicinity of the left external jugular vein were ligated at their junction with the external jugular. This created a blind pouch within the external jugular vein into which thoracic duct lymph flowed unrestricted and from which thoracic duct lymph could be obtained without performing a thoracotomy. A cannula, coated with tridecylmethylammonium heparinate-Heparin (Polysciences, Inc., Warrington, PA), was sutured into the isolated portion of the external jugular.

Anesthesia was discontinued prior to the administration of the isotopes (1.0 mcI of [15-3H]retinol and 250 μCi of [14C]cholesterol in 2.0 ml of corn oil), which were given by gastric intubation. The gastric tube was rinsed with 150 ml of Mocha Mix to which had been added sucrose (2.1% by weight) and cholesterol (0.2% by weight). The animals were alert within 30 min. The dogs were given an additional 300 ml of Mocha Mix with sucrose and cholesterol during a 1-h period after the administration of the radiolabeled compounds. Mocha Mix was made available to the dogs during lymph collection (some animals did drink during this time period). Mocha Mix (Presto Food Products, Industry, CA) is a liquid nondairy creamer that contains polyunsaturated fat (primarily soybean oil), and 74% of its calories are derived from fat. The high fat mixture with added sucrose and cholesterol was used to stimulate chylomicron production. Lymph was collected continuously in Fenwic blood packs (Travenol Laboratories, Inc., Deerfield, IL) containing 250 mg of disodium EDTA, 16,000 Kallikrein inactivator units of Trasylol (Mobay Chemical Company, New York, NY), and 167 mg of 5,5′-dithiobis-(2-nitrobenzoic acid) (Sigma) in 5 ml of H2O, pH 7.65, and affixed to each dog’s abdomen. Lymph was collected for 1-h periods, and, in most instances, the 4-5-h fractions were used to harvest the radiolabeled chylomicrons. Kanamycin (100 μg/ml), phenylmethylsulfonyl fluoride (1 mM; Sigma), and benzamidine (1 mM) were added to the lymph after collection. The inhibitors were those recommended by Edelstein and Scanut (34).

Lymph with the highest radioactivity was centrifuged at 28,000 rpm for 90 min in an SW 28 rotor (Beckman Instruments, Palo Alto, CA) at 20 °C. A thick, creamy layer composed of chylomicrons was removed, resuspended, passed through 0.45-μm filters (resulting in the loss of only 7% of [14C]cholesterol and 17% of [3H]retinol), stored at 4 °C, and used for in vivo studies within 2 weeks of preparation. The lipid composition of the canine chylomicrons is shown in Table I. More than 90% of the [3H]retinol and more than 70% of the [14C]cholesterol present in the chylomicrons were esterified. The particles were typically 1000-5000 Å in diameter, as determined by negative-staining electron microscopy.

To prepare canine [14C]cholesterol- and [3H]retinol-labeled chylomicrons enriched in apoE, the chylomicrons were incubated for 1 h at 37 °C in the presence or absence of rabbit apoE. Rabbit apoE was isolated and purified from the plasma of cholesterol-fed rabbits, as described previously (35). The apoE was added to the chylomicrons at a chylomicron cholesterol/apoE weight ratio of 0.04. The chylomicrons, with or without added apoE, were then injected into the recipient rabbits.

Rabbit lymph was obtained from the mesenteric duct (8). The lymph duct was cannulated after a 2-h feeding of Mocha Mix containing [3H]retinol and [14C]cholesterol.

Canine Chylomicron Remnant Preparation—Chylomicron remnants were prepared by injecting chylomicrons into hepatectomized rabbits. A functional hepatectomy was performed on methoxyflurane-anesthetized rabbits by ligating the superior and inferior mesenteric, celiac, and hepatic arteries. A ligature was also placed around the base of each lobe of the liver. Animals were infused through the femoral vein with 5% dextrose in saline throughout the study. Chylomicrons (150-300 mg of triglyceride/kg of body weight) were injected into the femoral vein and allowed to circulate for 30 or 120 min in these functionally hepatectomized animals. In all studies the amount of [3H]retinol and [14C]cholesterol detected in the liver was negligible (<0.01% of the injected dose). Blood was collected in EDTA, and the plasma was centrifuged in an SW 41 rotor at 41,000 rpm for 90 min or in an SW 28 rotor at 28,000 rpm for 2 h, 45 min to collect chylomicron remnants (Sv > 175). The modification of chylomicrons during circulation in hepatectomized rabbits was calculated from the decrease in the ratio of triglyceride (milligrams/ml) to cholesterol (milligrams/ml). The change in the triglyceride/cholesterol ratio very likely reflects the lipolytic hydrolysis of triglycerides and/or the transfer of cholesterol to the remnants. The ratio of triglyceride to cholesterol in chylomicrons is approximately 66:1 (w/w), whereas the ratio in chylomicron remnants obtained after 30 min of circulation in a hepatectomized rabbit is 26:1. Furthermore, after 120 min of circulation in a hepatectomized rabbit, the ratio of triglyceride to cholesterol is approximately 10:1 (Table I).

Canine and Rabbit d < 1.006 g/ml Lipoproteins—To prepare canine VLDL, 200 ml of blood was obtained from animals fasted overnight. The plasma was centrifuged in a Ti-60 rotor at 50,000 rpm for 16 h at 4 °C. The d < 1.006 g/ml lipoproteins were washed once and concentrated by ultrafiltration (PM-30 membrane, Amicon Corp., Lexington, MA). The lipid composition of the VLDL was 76.6% triglyceride, 9.3% cholesterol, and 14.1% phospholipid. Rabbit d < 1.006 g/ml lipoproteins were prepared similarly, except that blood was obtained from either rabbits fasted overnight or from rabbits fed a high-fat diet. Mocha Mix (fed 4.5 h before the blood sample was obtained).

Canine β-VLDL and HDL—The coconut oil-cholesterol-fed dogs were fasted overnight, and blood was collected in chilled tubes containing disodium EDTA (0.01% w/v) final concentration; pH 7.4. All procedures involving lipoprotein isolation and characterization were started immediately after the blood drawing and were carried out at 4 °C. The d < 1.006 g/ml fraction from cholesterol-fed dogs was isolated at plasma density and washed in 0.15 M NaCl (d = 1.006 g/ml) at 50,000 rpm in a Ti-60 rotor. To prepare canine β-VLDL, the washed d < 1.006 g/ml fraction (≈15 mg of lipoprotein protein) was subjected to Pevikon block electrophoresis (Pevikon Corp., Yonkers, NY) (31, 36, 37). The location of the pre-β and β bands was visualized by using an ultraviolet light, and the β-VLDL band was removed from the block and eluted with saline. This fraction was then concentrated with PM-30 membrane and used for further characterization.
The lipid composition of the β-VLDL was 21.3% triglyceride, 54.5% cholesterol, and 24.2% phospholipid. The apoE-HDL (d = 1.006-1.02 g/ml) were isolated from cholesterol-fed dogs by a combination of ultracentrifugation and Pevikon block electrophoresis as described previously (31, 37). These cholesterol-rich lipoproteins contain less than 1% of their chemical composition as triglyceride (31).

**Chylomicron Metabolism in Rabbits**

After the injection of radiolabel in corn oil followed by 20 ml of Mocha Mix (containing 2.1% sucrose and 0.2% cholesterol) via gastric intubation. Alternatively, the rabbits were given the radiolabel in corn oil followed by 20 ml of Rabbit Chow finely ground and suspended in water. At various time periods after ingestion of the radiolabeled retinol and cholesterol, the animals were killed, and tissues were collected as described.

**Extraction of Lipids from Tissues**

For extraction and quantitation of [3H]retinol and [14C]cholesterol, tissues (0.1–0.5 g) were digested in 0.5 ml of 6 N KOH (prepared freshly by mixing 2 volumes of 9 N KOH and 1 volume of saline-EDTA) at 37°C until completely solubilized. Then, 0.5 ml of 100% ethanol was added to the solubilized tissue and vortexed. Lipids were then extracted twice into 6.0 ml of hexane. The top hexane layers were collected after centrifugation (3000 rpm for 10 min), dried under N2, and suspended in 0.5 ml of 100% ethanol and 10 ml of non-aqueous scintillation mixture (Beckman) before scintillation counting.

To quantitate radiolabeled lipids in the entire rabbit skeleton, all the bones were cut into pieces, placed in a Soxhlet apparatus, and subjected to extraction with excess chloroform/methanol (2:1, v/v) (40). Extraction was performed four times, each time for 24 h. The chloroform/methanol was evaporated, and then the lipids were dissolved in 100% ethanol and counted in the presence of non-aqueous scintillation mixture.

**Electron Microscopy**

For ultrastructural studies of bone marrow, the anesthetized rabbits were perfused at 80 mm of mercury through the left ventricle with 500 ml of modified Eagle’s medium (GBCO), followed by 500 ml of 5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.3. Bone marrow samples obtained from the femur were subjected to postfixation for 2–4 h in 0.05 M sodium barbital containing 2% OsO4, 2% K2Fe(CN)6, and 5% sucrose (41). The en bloc staining was performed overnight in 2% uranyl acetate. The tissues were then dehydrated with acetone and embedded in Araldite (150A LK51, Whatman, Clifton, NJ) (41). Thin sections were examined by a JEOL electron microscope (model 100CXII).

**Lipid Analysis**

The concentrations of lipoprotein cholesterol, triglyceride, and phospholipid were determined as described (42). Retinol was extracted from the plasma or individual lipoprotein fractions by the addition of equal volumes of 100% ethanol. Free [3H]retinol and esterified [3H]retinol were separated by high performance liquid chromatography (Ultrasphere ODS, 5 μm, 4.6-mm × 25-cm column, isocratic elution with 100% ethanol at 1.0 ml/min flow rate) (43). The percentage of [3H]retinol and [14C]cholesterol content was determined after the separation of cholesterol and cholesteryl ester on a thin-layer plate (150A LK51, Whatman, Clifton, NJ) by using 80 ml of hexane, 20 ml of diethyl ether, and 1.0 ml of NH4OH solvent system (42). The lipids were visualized by iodine vapors, scraped into scintillation vials, and counted.

**Data Analysis**

Calculation of the percent of injected dose of chylomicron remaining in the rabbit plasma at various time intervals was based on the estimate that plasma volume constituted 3.5% of body weight. For all
tissues, except the bone marrow, the uptake of radiolabeled lipoproteins/g was calculated by dividing the disintegrations/min by the actual wet weight of each tissue used for extraction and/or counting, and calculation of the uptake per organ was based on the actual organ weights at the end of the experiment. The bone marrow uptake of radiolabeled lipoproteins/g represented the average values obtained for the marrow from the femur and tibia. The calculation of bone marrow uptake per organ was based on the estimate that the bone marrow constituted 2.2% of the total body weight (44-46). Unless stated otherwise, the data represent the percent of injected dose.

RESULTS

Chylomicron Clearance by Liver, Bone Marrow, and Spleen—Canine thoracic duct chylomicrons labeled in vivo with [14C]cholesterol and [3H]retinol were injected intravenously into normal fasted rabbits, and the plasma clearance and the uptake by specific tissues were examined. Twenty minutes after injection, 75 and 78% of the [14C]cholesterol- and [3H]retinol-labeled chylomicrons were cleared from the plasma, respectively (Fig. 1A, top panel). Analysis of the tissue-specific uptake of the radiolabeled chylomicrons based on percent of injected dose/g of tissue revealed that the spleen, bone marrow, and liver were responsible for the greatest uptake (Fig. 1B). Other tissues each possessed less than 0.12% of the injected dose/g wet weight. On the other hand, when the uptake was calculated per organ, the liver and bone marrow accounted for the greatest uptake (Fig. 1C). Twenty minutes after injection the liver had accumulated 34.4% of the [14C]cholesterol- and 26.8% of the [3H]retinol-labeled chylomicrons. The bone marrow possessed 29.3% of the [14C]cholesterol and 13.8% of the [3H]retinol at 20 min. In contrast, the spleen, which is small in the rabbit, was responsible for the clearance of 2.4% of the injected chylomicrons. The other organs (kidney, lung, adrenal, heart, and adipose tissue) each contained less than 1% of the injected dose at 20 min (Fig. 1C). In this study, the recovery of [14C]cholesterol and [3H]retinol, including the amount remaining in the plasma plus that recovered in the various tissues, was 93 and 71%, respectively. Of the radiolabeled lipoproteins cleared from the plasma, the liver and the bone marrow together accounted for 85% of the [14C]cholesterol and 58% of the [3H]retinol.

The data shown in Fig. 1 are from a representative study. In six experiments in which the dose of chylomicrons was 24-76 mg of triglyceride/kg of body weight, the percent of the injected dose cleared from the plasma at 20 min ranged from 66 to 79% for [14C]cholesterol and 66 to 80% for [3H]retinol. The liver accounted for the uptake of 22-54% of the [14C]cholesterol- and 14-36% of the [3H]retinol-labeled chylomicrons (based on percent of injected dose/organ at 20 min). The bone marrow accounted for the uptake of 20-43% of [14C]cholesterol- and 18-46% of [3H]retinol-labeled chylomicrons. Recovery of the radiolabel in the plasma plus the amount in the tissues ranged from 93 to 117% for [14C]cholesterol and 71 to 100% for [3H]retinol. The recovery of [3H]retinol was usually lower than that of [14C]cholesterol at 20 min. However, recovery of [14C]cholesterol and [3H]retinol ranged from 98 to 101% in two studies in which tissue distribution was assessed 5 and 10 min after chylomicron injection.

To determine the dose response for the uptake of chylomicrons by liver and bone marrow, various amounts of chylomicron cholesterol (0.07-20 mg of chylomicron cholesterol/kg of body weight; 0.2-40 mg/rabbit) were injected. The triglyceride concentrations injected ranged from 6 to 615 mg/kg of body weight. The distribution of [14C]cholesterol in the plasma, liver, and bone marrow was determined at 20 min in the recipient rabbits. Because of progressive lipolytic processing and modification of the chylomicrons as they circulated, uptake of the lipoproteins by the tissues was based on the cholesterol concentration that could be deduced from the specific activity of [14C]cholesterol in the chylomicrons injected. As shown in Fig. 2, as the quantity of chylomicrons injected was increased, the percent of the injected dose cleared from the plasma decreased. For example, at a concentration of 0.07 mg of chylomicron cholesterol/kg of body weight, 93% of the injected dose was cleared; however, when 20 mg of chylomicron cholesterol/kg of body weight was injected, only

![Graph A: Plasma clearance and tissue uptake of canine chylomicrons in a normal rabbit. An unanesthetized rabbit, fasted overnight, was injected with chylomicrons (containing 48.5 mg of triglyceride and 8 mg of cholesterol/kg of body weight) isolated from thoracic duct lymph by ultracentrifugation. Twenty minutes after injection of the [14C]cholesterol- and [3H]retinol-labeled chylomicrons, the rabbit was euthanized and tissues were collected, rinsed twice in ice-cold saline, blot-dried, and weighed. Two pieces of each tissue (0.05-2.0 mg) were used for extraction of lipids. Panel A, the plasma clearance of the chylomicrons. Panel B, the percent of injected dose taken up per g wet weight of several tissues. Panel C, the percent of the injected dose taken up per organ.](image-url)
44% of the dose was removed from the plasma. At the lower concentrations (Fig. 2A), the liver was the major organ responsible for chylomicron uptake, whereas at higher concentrations (Fig. 2B) the bone marrow was responsible for uptake of a larger amount (i.e. about equal to that of the liver). For example, when 0.3 mg of chylomicron cholesterol/kg of body weight was injected, only 1.3 µg of cholesterol remained in 1 ml of plasma, and 8.2 and 3.2 µg were present in 1 g of liver and bone marrow, respectively. In contrast, when 6.6 mg of chylomicron cholesterol was injected, 68.8 µg of cholesterol remained in 1 ml of plasma, and 44.2 and 58.3 µg of cholesterol were present in each gram of liver and bone marrow, respectively. It appears that uptake by both organs reaches saturation at the higher concentrations (Fig. 2B).

To determine the fate of canine chylomicrons taken up by the bone marrow, the accumulation of [14C]cholesterol and [3H]retinol was studied at different times. Three rabbits were injected with radiolabeled chylomicrons, killed at 20, 120, and 240 min, and the plasma, liver, and bone marrow were analyzed (Fig. 3). The amount of [14C]cholesterol taken up by the bone marrow was rather constant (~20-25% of the injected dose) at these intervals. However, the percent of [3H]retinol decreased with time. These results suggested that the chylomicrons were catabolized in the bone marrow, with the [14C] cholesterol being retained and the [3H]retinol being transferred from the marrow. Presumably, the [3H]retinol is redistributed to the liver for storage or to other cells for utilization.

To determine the tissue distribution of chylomicron uptake in the absence of the liver, canine thoracic duct chylomicrons were injected into hepatectomized rabbits and their fate analyzed at 30 min. Approximately 50% of the radiolabeled chylomicrons were cleared from the plasma of the hepatectomized rabbit (Fig. 4A). Analysis of the tissues revealed that bone marrow was responsible for the uptake of 33% of [14C] cholesterol- and 32% of [3H]retinol-labeled chylomicrons (Fig. 4B). The lung accounted for approximately 5% of the uptake of the radiolabel, whereas the spleen, kidney, adrenals, and heart accumulated less than 0.5% of the injected radiolabeled lipoproteins. These results highlighted further the specificity of the uptake of chylomicrons by the bone marrow.

The bone marrow took up not only chylomicrons, but chylomicron remnants also. When the chylomicron remnants were isolated from the plasma of the hepatectomized rabbit and reinjected into a normal fasted rabbit, the liver and the bone marrow accounted for the greatest uptake (Fig. 5B). The triglyceride/cholesterol ratio was 90:1 in the chylomicrons before injection into the hepatectomized rabbit and decreased to 50:1 after 30 min of circulation. The reinjected chylomicron remnants were rapidly cleared from the plasma (82 and 87%, respectively, of the injected [14C]cholesterol and [3H]retinol dose at 20 min) (Fig. 5A), and 45.5% of the [14C]cholesterol- and 40.2% of the [3H]retinol-labeled lipoproteins were present in the liver (Fig. 5). A significant amount of label (23.2 and 19.8% of the [14C]cholesterol and [3H]retinol, respectively) was found in the bone marrow. The lung contained 3.4 and 1.5% of [14C]cholesterol and [3H]retinol. Other tissues contained less than 1% of the injected dose.

Validation of Results Demonstrating Chylomicron Uptake by Bone Marrow—Several possible artifacts that might account for the uptake of chylomicrons by the bone marrow were considered and eliminated. First, it is possible that the radioactivity within the bone marrow of the femur and tibia might not reflect uptake by the bone marrow of other regions of the body, such as the sternum and the iliac crest. However, the percent of injected dose of [14C]cholesterol recovered per g of bone marrow taken from the femur, tibia, sternum, and iliac crest was 0.34, 0.29, 0.35, and 0.31, respectively, in a normal rabbit 20 min after injection of the radiolabeled chylomicrons. Similarly, the percent of injected dose of [14C] cholesterol recovered per g of the bone marrow of femur, tibia, and sternum was 0.43, 0.34, and 0.45, respectively, in a he-
Chylomicron Metabolism in Rabbits

A

[3H]retinol
[14C]cholesterol

B

[3H]retinol
[14C]cholesterol

Liver Bone Marrow Spleen Kidney Lung Heart

FIG. 4. Plasma clearance and tissue uptake of canine chylomicrons in hepatectomized rabbit. Chylomicrons (containing 152 mg of triglyceride/kg of body weight) were injected in the femoral vein of a hepatectomized (described under "Materials and Methods") rabbit. Plasma samples were withdrawn at designated time points from a femoral artery of the other leg, and the tissues were processed as described under "Materials and Methods" and Fig. 1. Panel A, the plasma clearance of [14C]cholesterol and [3H]retinol. Panel B, uptake of [14C]cholesterol and [3H]retinol in different tissues or remaining in the plasma 30 min after injection of the radiolabeled chylomicrons.

Hepatectomized rabbit 30 min after injection of chylomicrons. These results demonstrated a similar extent of chylomicron uptake by the bone marrow in different regions of the body.

Second, it is possible that the bone marrow uptake was overestimated as a result of using a factor of 2.2% to calculate the percent of body weight represented by the bone marrow. However, it had been rigorously established in rabbits (44-46) and in other animals (47-51) that the bone marrow represents approximately 1.75-2.6% of the total body weight. To measure directly the uptake of radiolabeled chylomicrons by the bone marrow, a rabbit was perfused with medium, all radiolabel was extracted from the bones with chloroform/methanol, and [14C]cholesterol recovery was determined as described under "Materials and Methods." Although it is recognized that direct extraction of radiolabel from bones may not be complete, this approach was considered to be the most direct way to prove that a significant quantity of chylomicrons was, in fact, taken up by the bone marrow. The rabbit was injected with 60 mg of triglyceride/kg of body weight. The process of extraction from the entire skeleton resulted in the

FIG. 5. Plasma clearance and tissue uptake of canine chylomicron remnants in normal rabbits. Chylomicron remnants were prepared by injection of chylomicrons intravenously into a hepatectomized rabbit, allowed to circulate for 30 min, and then isolated from the plasma by ultracentrifugation (see "Materials and Methods"). The chylomicron remnants were reinjected into a normal fasted rabbit at a concentration of 49.3 mg of triglyceride and 1.5 mg of cholesterol/kg of body weight. Panel A, the plasma clearance at 20 min. Panel B, the tissue uptake of chylomicron remnants at 20 min.

recovery of approximately 13% of the injected dose. In four other experiments in which the rabbits received 60-64 mg of triglyceride/kg of body weight, the bone marrow accounted for the uptake of 16, 20, 23, and 40% of the injected dose based upon the factor of 2.2%. Therefore, we conclude that the use of the 2.2% factor provides a reasonable estimate of bone marrow uptake.

Third, consideration was also given to the possibility that the uptake of chylomicrons resulted from an alteration in the particles during the flotation by centrifugation or during removal and resuspension of the floated chylomicrons. Although this possibility is extremely difficult to evaluate, we attempted to do so by preparing chylomicrons in three different ways. Whole canine lymph containing radiolabeled chylomicrons (never centrifuged), lymph chylomicrons centrifuged and immediately resuspended in the lymph without being isolated, and lymph chylomicrons isolated by centrifugation and resuspended in saline were each injected into a rabbit. Plasma clearance and tissue distribution of the [14C] cholesterol and [3H]retinol were determined 20 min after injection. As shown in Table II, the plasma clearance and tissue distribution were remarkably similar. When rabbit
variation is taken into account, it is clear that chylomicron clearance and tissue distribution were not grossly different regardless of whether or not the chylomicrons were isolated. In addition, as will be shown later, chylomicron synthesis induced by dietary fat results in the appearance of intestinally absorbed [14C]cholesterol and [3H]retinol in the bone marrow in vivo. This further substantiates the conclusion that chylomicron uptake by the bone marrow is not due to an alteration in the particles.

Fourth, consideration was also given to the possibility that the mode of injection might alter the tissue distribution. In all other studies described, the lipoproteins were given as a bolus injection. To mimic a more natural entry of intestinal lipoproteins into the plasma, whole thoracic duct lymph labeled in vivo with [14C]cholesterol was slowly infused intravenously into a rabbit at a low concentration over a period of 60 min, and the tissue distribution of the [14C]cholesterol was determined 120 min after the infusion period. The rabbit was infused at a rate of 1.7 mg of whole lymph triglyceride/min/kg of whole body weight (total triglyceride was 246 mg).

Approximately 6% of the injected dose of the [14C]cholesterol in the lymph remained in the plasma 120 min after the infusion. The [14C]cholesterol uptake by the liver and the bone marrow was 54 and 27% of the injected dose, respectively. The lung contained 1% of the injected dose, whereas the spleen, kidney, adrenal, and heart had less than 1%.

It is also possible that the radioactivity in the bone marrow reflected gross blood contamination or nonspecific trapping of labeled chylomicrons. However, this was ruled out by results obtained with single hind limb perfusion studies designed to remove blood or trapped chylomicrons, or both. The nonperfused contralateral hind limb served as a control. The single-femur perfusions resulted in the loss of the gross appearance of blood in the bone marrow; however, even with extensive perfusion, there was only a small loss of the radio-labeled chylomicrons from femoral bone marrow (Table III). Furthermore, there was no further significant loss when the hind limb was perfused with Krebs-Ringer bicarbonate containing 10–100 units of heparin/ml. These studies show that the uptake of chylomicrons in the bone marrow is not affected by perfusion and is not reversible with heparin, suggesting that the chylomicrons were irreversibly bound to or taken up by cells in the bone marrow.

**Uptake of Chylomicrons by Perisinusoidal Macrophages in the Bone Marrow**—To identify the cell type responsible for the uptake of chylomicrons by the bone marrow, rabbits were perfused with glutaraldehyde 20 min after the injection of chylomicrons, and marrow from the femur was examined by electron microscopy. As shown in Fig. 6, electron microscopy revealed an abundance of chylomicron-sized lipoprotein particles within the resident perisinusoidal macrophages in the bone marrow. By measuring at intervals after injection of the chylomicrons, it was possible to demonstrate the loss of recognizable chylomicrons in the cells and the presence of amorphous material within the lysosomes of these cells, a result consistent with internalization and degradation of the lipoproteins by the macrophages. Chylomicrons could not be identified within the perisinusoidal macrophages of bone marrow obtained from a fasted rabbit that had not received an injection of chylomicrons.

**Specificity of Bone Marrow Uptake of Lipoproteins**—Chyl-

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**TABLE II**

Effect of centrifugation on chylomicron uptake by liver and bone marrow

<table>
<thead>
<tr>
<th>Treatment of lymph</th>
<th>% of injected dose at 20 min*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>[H]</td>
</tr>
<tr>
<td>Lymph*</td>
<td>30.2</td>
</tr>
<tr>
<td>Centrifuged and resuspended lymph</td>
<td>28.1</td>
</tr>
<tr>
<td>Isolated chylomicrons a</td>
<td>33.0</td>
</tr>
</tbody>
</table>

*Percent of injected dose of [14C]cholesterol- and [3H]retinol-labeled lipoproteins remaining in the plasma and taken up by the liver and bone marrow at 20 min. Data represent the average of values obtained for two rabbits in each experiment.

Rabbits were injected with 10 ml of whole canine lymph (no centrifugation).

Rabbits were injected with 10 ml of canine lymph that had been centrifuged at 28,000 rpm in an SW 28 rotor for 90 min to float the chylomicrons; however, the chylomicron fraction was not removed but simply resuspended in the lymph.

Rabbits were injected with chylomicrons that had been isolated by ultracentrifugation, removed, and resuspended in saline (10 ml total volume).

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**TABLE III**

Effect of perfusions on the release of chylomicrons from the rabbit femur

<table>
<thead>
<tr>
<th>Perfusion conditions a</th>
<th>[14C]Cholesterol Nonperfused Perfused</th>
<th>[3H]Retinol Nonperfused Perfused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krebs-Ringer bicarbonate buffer, pH 7.4 (500 ml)</td>
<td>25.8</td>
<td>25.0</td>
</tr>
<tr>
<td>Krebs-Ringer bicarbonate buffer, pH 7.4 (250 ml) + Krebs-Ringer bicarbonate buffer containing 10 units of heparin/ml (250 ml)</td>
<td>25.0</td>
<td>24.5</td>
</tr>
<tr>
<td>Krebs-Ringer bicarbonate buffer (250 ml) + Krebs-Ringer bicarbonate buffer containing 100 units of heparin/ml (250 ml) + Krebs-Ringer bicarbonate buffer (250 ml)</td>
<td>31.6</td>
<td>24.4</td>
</tr>
</tbody>
</table>

*Values represent the percent of the injected dose of radiolabeled chylomicrons taken up by the bone marrow of the nonperfused and the perfused hind limb (calculated per organ).

*When more than one medium was used, the perfusions were performed in the succession given. The amount of medium used is stated in parentheses.
the bone marrow were compared with the uptake of VLDL through endothelial cells. Cellular processes extending into the blood sinus were commonly seen in the rabbit bone marrow.

As expected, recovery of the \(^{125}\)I (approximately 50–85% in plasma and tissues) was not as complete as with \(^{14}\)C cholesterol- or \(^{3}H\) retinol-labeled lipoproteins, presumably because of the loss of free \(^{125}\)I or small \(^{125}\)I-labeled peptides generated during lipoprotein catabolism. However, the consistency of the results indicated that the larger, triglyceride-rich lipoproteins were specifically taken up by the bone marrow as contrasted to the more cholesterol-rich \(\beta\)-VLDL and apoE-HDL. The uptake of a small fraction of \(\beta\)-VLDL by the bone marrow in a hepatectomized rabbit may reflect the presence of low levels of larger, triglyceride-rich particles in this fraction.

Bone Marrow Uptake of Rabbit Lipoproteins—To investigate further the specificity of uptake of lipoproteins by rabbit bone marrow, rabbit mesenteric lymph chylomicrons were obtained. The rabbit lymph was collected in two different ways: collected with EDTA plus added proteolytic inhibitors and an antibiotic (the procedure used for canine lymph collection; see "Materials and Methods"), or without anticoagulant and proteolytic inhibitors (8). Whole lymph was injected directly into the animal, and the \(^{3}H\) retinol accumulation in the liver and the bone marrow was measured after 20 min. There was no major difference in the plasma clearance and
tissue distribution of rabbit lymph chylomicrons versus the canine lymph chylomicrons. In addition, the method of collection did not obviously alter the tissue distribution. With the lymph obtained by using inhibitors, 75% of the [3H]retinol-labeled lipoproteins were cleared from the plasma at 20 min and 93% and 17% of the [3H]retinol were accounted for in the liver and the bone marrow, respectively, and with the lymph collected without using inhibitors, 65% of the [3H]retinol-labeled lipoproteins were cleared from the plasma, and 33% and 18% of the [3H]retinol were accounted for in the liver and bone marrow, respectively. Likewise, we have shown that the presence or absence of inhibitors does not alter the uptake of radiolabeled canine lymph chylomicrons by the bone marrow (data not shown).

To determine whether the bone marrow uptake of intestinally synthesized lipoproteins occurs in vivo, rabbits were fed [14C]cholesterol and [3H]retinol in corn oil mixed with the polyunsaturated fat-rich Mocha Mix or mixed with a slurry of Rabbit Chow (see "Materials and Methods" for details). Intestinally absorbed cholesterol and retinol are known to be transported into the circulation by chylomicrons, and therefore the tissue distribution of the cholesterol and retinol would be expected to reflect the in vivo metabolism of chylomicrons. However, it is recognized that the [14C]cholesterol could be reutilized for endogenous lipoprotein production and that the [3H]retinol could be redistributed to storage sites. Regardless of these potential complications, this approach should give at least a qualitative estimate of the in vivo sites of chylomicron metabolism. The tissue distribution of the [14C]cholesterol and [3H]retinol, 3.5 h after the rabbit was fed the corn oil-Mocha Mix, revealed that the three major organs accumulating the label were the liver, spleen, and bone marrow, based on radiolabel/g of tissue (Fig. 8A). Calculated on a per organ basis, the liver and the bone marrow were by far the major tissues accumulating the radiolabel (Fig. 8B). Similar results were obtained when the [14C]cholesterol and [3H]retinol were administered along with Rabbit Chow (without the added Mocha Mix) (see below). Thus, these studies indicated that native dietary lipoproteins were cleared primarily by the liver and the bone marrow and that the distribution was similar to that observed for intravenously injected canine lymph chylomicrons (compare Fig. 1 with Fig. 8).

The uptake of intestinally absorbed [14C]cholesterol by the liver and the bone marrow was further analyzed as a function of time after ingestion and type of diet (Table IV). There was significant animal variation with respect to the amount of [14C]cholesterol absorbed, which is not unexpected; however, in all animals there was significant uptake of intestine-derived lipoproteins by the bone marrow. The ratio of [14C]cholesterol accumulation in the liver to that in the bone marrow varied from 1.3 to 5.0 (Table IV).

To determine the uptake of other rabbit lipoproteins by the liver and the bone marrow, 131I-labeled VLDL (d < 1.006 g/ml) obtained from fasted or fed rabbits were injected into fasted rabbits. When VLDL obtained from a fasted animal were injected into a normal rabbit, approximately 24% of the injected dose was cleared from the plasma at 20 min, and 12 and 4% of the label were accounted for in the liver and the bone marrow, respectively. The d < 1.006 g/ml lipoproteins from a fed rabbit (presumably containing some remnant lipoproteins) were cleared somewhat more rapidly, and 37% of the dose was cleared from the plasma at 30 min. The liver and the bone marrow contained 12 and 8%, respectively, of the injected dose at this time. These data indicate that triglyceride-rich rabbit particles are also taken up by the bone marrow, which is consistent with results observed when using

![Figure 8. Postprandial tissue distribution of [3H]retinol and [14C]cholesterol. A rabbit was fed (by gastric intubation) [3H]retinol (100 µCi) and [14C]cholesterol (25 µCi) in 0.5 ml of corn oil followed by 20 ml of Mocha Mix containing 2.1% sucrose and 0.2% cholesterol. At 3.5 h after feeding the rabbit was perfused and the radioactivity determined in different tissues as described under "Materials and Methods" and in Fig. 1. Panel A, uptake of [14C]cholesterol and [3H]retinol (disintegrations/min)/g of wet tissue, and the amount in 1 ml of plasma. Panel B, radiolabel/organ.]

<table>
<thead>
<tr>
<th>Feeding conditions</th>
<th>Analysis time post-feeding</th>
<th>Liver Bone marrow Liver/bone marrow ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil + Mocha Mix</td>
<td>3.0</td>
<td>27.2</td>
</tr>
<tr>
<td>Corn oil + Rabbit Chow</td>
<td>4.5</td>
<td>161.2</td>
</tr>
<tr>
<td>Corn oil + Rabbit Chow</td>
<td>6.0</td>
<td>110.0</td>
</tr>
<tr>
<td>Corn oil + Rabbit Chow</td>
<td>6.0</td>
<td>58.7</td>
</tr>
</tbody>
</table>

*Only the [14C]cholesterol values (disintegrations/min) are reported because of the rather rapid loss of [3H]retinol from the bone marrow in contrast to the apparent accumulation of the [14C]cholesterol (see Fig. 3).
triglyceride-rich canine lipoproteins.

Modulation of Chylomicron Uptake by Liver and Bone Marrow—As already described, the liver takes up a larger fraction of chylomicrons when they are injected at low concentration and the uptake is decreased at higher concentration of chylomicrons. Studies were undertaken to identify other factors that might modulate the tissue-specific uptake of chylomicrons by both the liver and the bone marrow. Chylomicrons were compared with chylomicron remnants. In addition, consideration was given to the possibility that the availability of apoE might be rate-limiting at higher concentrations of chylomicrons or chylomicron remnants.

Chylomicron remnants were prepared by injecting chylomicrons in hepatectomized rabbits and isolating the remnants (Sf > 175) by centrifugation after 30 and 120 min. During circulation, the triglyceride/cholesterol ratio changed from approximately 26:1 at 30 min to approximately 10:1 at 120 min. For comparison, the chylomicrons, before injection into the hepatectomized rabbit, had a triglyceride/cholesterol ratio of 44:1. The radiolabeled canine chylomicrons and chylomicron remnants were injected into normal fasted rabbits, and the [14C]cholesterol distribution in liver, bone marrow, and plasma was determined. At 20 min after injection of the chylomicrons, 39% of the injected dose was in the liver and 18% in the bone marrow (Fig. 9). In contrast, the chylomicron remnants were more rapidly cleared by the liver (61% of the injected dose of remnants generated by 30 min of circulation in a hepatectomized rabbit, and 73% of the injected dose of remnants generated by 120 min of circulation) (Fig. 9). The percent of the injected dose accumulating in the bone marrow was 13 and 9% for the two types of chylomicron remnants (Fig. 9). Chylomicron remnants were preferentially cleared from the plasma by the liver: however, there was still significant uptake of chylomicron remnants by the bone marrow.

To study the effect of added apoE on chylomicron metabolism, canine chylomicrons were incubated with or without rabbit apoE for 1 h at 37°C and were then injected into normal fasted rabbits at a chylomicron dose of 0.54 mg of cholesterol/kg of body weight (49 mg of triglyceride). The ratio of cholesterol to added apoE was 0.04 by weight. Fig. 10 shows the plasma clearance curves and uptake of [14C]cholesterol by the liver and the bone marrow in these rabbits. Addition of apoE resulted in a more rapid clearance of chylomicrons from the plasma and increased uptake by the liver, whereas uptake by the bone marrow was slightly decreased. At 20 min, 70% of the injected dose of [14C]cholesterol was cleared from the plasma of the rabbit injected with chylomicrons; however, the amount of chylomicrons cleared from the plasma increased to 89% of the injected dose for chylomicrons incubated with apoE. The uptake of [14C]cholesterol by the liver was 0.34% and 45% of injected dose for chylomicrons in the presence and absence of added apoE, respectively. The uptake of chylomicrons by the bone marrow was 21% and 27% of injected dose of [14C]cholesterol, respectively.

Fig. 9. Comparison of tissue uptake of canine chylomicrons and chylomicron remnants 20 min after injection. Remnants were generated by circulation of chylomicrons in hepatectomized rabbits for 30 or 120 min. The following doses were injected into normal fasted rabbits: chylomicrons (containing 15 mg of triglyceride and 0.54 mg of cholesterol/kg of body weight), chylomicron remnants generated in 30 min (containing 21 mg of triglyceride and 0.78 mg of cholesterol/kg of body weight), and chylomicron remnants generated in 120 min (containing 7 mg of triglyceride and 0.71 mg of cholesterol/kg of body weight).

Fig. 10. Effect of rabbit apoE on plasma clearance and tissue uptake of chylomicrons. Canine chylomicrons with and without added rabbit apoE were injected at a dose of 49 mg of triglyceride and 0.54 mg of cholesterol/kg of body weight. Panel A, the plasma clearance of these chylomicrons. Panel B, the tissue distribution of these chylomicrons at 20 min.

DISCUSSION

Canine thoracic duct and rabbit mesenteric lymph chylomicrons labeled in vivo with [14C]cholesterol and [3H]retinol were rapidly cleared from the plasma of normal rabbits and appeared almost quantitatively within the liver and the bone marrow. Approximately two-thirds to three-fourths of the canine chylomicrons were cleared from the plasma within 20 min. The rapid clearance of chylomicrons from plasma is consistent with all previous chylomicron metabolism studies in rabbits (8, 26, 27, 52). Depending upon the particular experiment and the dose of chylomicron triglyceride injected, we typically found that 50–60% of the radiolabeled chylomicrons cleared from the plasma were in the liver (see Fig. 1C). However, analysis of a large number of tissues indicated that a large percent (typically 20–40%) of the chylomicrons cleared from the plasma by 20 min was found in the bone marrow (Fig. 1C). At lower chylomicron concentrations a larger fraction of particles cleared from the plasma appeared in the liver, whereas at higher chylomicron concentrations more were detected within the bone marrow. However, in all studies there was significant uptake by the bone marrow.
The finding that the liver was not the only major organ to take up radiolabeled chylomicrons is consistent with the studies of Ross and Zilversmit (26), who reported a recovery of about 56–60% of the injected \(^{14}C\)-labeled cholesteryl and \(^3H\)-labeled retinyl esters of rabbit lymph chylomicrons within the liver. They concluded that in rabbits a substantial portion of chylomicrons was removed by extrathoracic tissues (26). However, these results differ from other reports (8, 52). Kita et al. (52) demonstrated that 91% of radiolabeled chylomicrons cleared from the plasma in 60 min were recovered in the liver, and Redgrave (8) demonstrated that 81% of chylomicrons cleared from the plasma at 15 min were in the liver. The apparent discrepancy between the present study and that of Kita et al. (52) may be explained by the type of chylomicrons studied. They used small chylomicrons produced by glucose feeding, in contrast to the use of large chylomicrons \((S_1 > 400)\) induced by fat feeding in this study. It is known that small chylomicrons (or intestinal VLDL) differ from large chylomicrons \((S_1 > 400)\) in their chemical composition (7, 53), their uptake by cultured cells (54, 55), and their in vivo catabolism (56).

Evidence that the liver was not the only organ capable of clearing chylomicrons was obtained by using heparotomized rabbits. Approximately 50% of the radiolabeled chylomicrons were cleared from the plasma of a heparotomized rabbit in 30 min, and the majority of the cleared chylomicrons could be accounted for in the bone marrow. This is consistent with the observation of Ross and Zilversmit (26), who reported that 43% of radiolabeled chylomicrons were cleared from heparotomized rabbits in 1 h; however, they did not quantify the amount within the bone marrow.

The mechanism responsible for the accumulation of radiolabeled chylomicrons in the bone marrow was investigated. Extensive perfusion studies revealed that the uptake was not readily reversible and suggested that the particles were sequestered intracellularly. Electron microscopic studies revealed that the perisinusoidal bone marrow macrophages were responsible for the uptake and accumulation of large quantities of chylomicrons. In contrast, chylomicrons were observed in the space of Disse and parenchymal cells of the liver but not in Kupffer's cells, which is consistent with the results of Stein et al. (57) and Jones et al. (58). The perisinusoidal macrophages in rabbit bone marrow protrude from the endothelium and phagocyte circulating red blood cells (59). Therefore, these cells have access to circulating lipoproteins. Nagata and Zilversmit (27) have shown that ethyl oleate treatment, used to block reticuloendothelial function, resulted in 60–70% recovery of newly absorbed cholesterol and retinol in plasma and liver. They have speculated that this might be due to the suppression of the phagocytic activity in the peripheral tissues. The present study shows that the peripheral tissue involved in chylomicron catabolism is the resident perisinusoidal macrophage. The potential of macrophages to metabolize chylomicrons and other triglyceride-rich lipoproteins has been shown in vitro by Gianturco et al. (60, 61). Furthermore, macrophages in bone marrow and xanthoma have been shown to be loaded with lipids and lipoproteins in patients with types I, III, and V hyperlipoproteinemia (62) and familial lipoprotein lipase deficiency (63).

Numerous control experiments were performed to rule out the effect of chylomicron isolation on uptake by the bone marrow. Whole lymph (never subjected to isolation procedures) or isolated chylomicrons prepared in several different ways consistently gave results that confirmed that both the liver and the bone marrow were major sites of chylomicron catabolism. The uptake of canine chylomicrons by bone marrow macrophages was not affected by the storage of lymph at room temperature with or without inhibitors for 2 days, storage at 4 °C, centrifugation and resuspension, or isolation of chylomicrons after centrifugation. In addition, screening of chylomicrons in a rabbit for 5 min and then immediately reintroducing whole blood into a second rabbit demonstrated that the liver and the bone marrow of the second animal cleared virtually the same quantity of \[^{14}C\]cholesterol and \[^{3}H\]retinol.

Furthermore, consideration was given to the possibility that the canine chylomicrons were behaving differently from rabbit chylomicrons. This was shown not to be the case by the fact that rabbit mesenteric lymph chylomicrons, prepared in the presence and absence of proteolytic inhibitors, were taken up in significant amounts by rabbit bone marrow. In fact, it was possible to demonstrate that \[^{14}C\]cholesterol and \[^{3}H\]retinol fed directly to a rabbit, along with either Mocha Mix or Rabbit Chow, resulted in their accumulation within both the liver and the bone marrow.

The uptake by rabbit bone marrow macrophages has been shown to be specific for triglyceride-rich lipoproteins. The bone marrow cleared chylomicrons, chylomicron remnants, and VLDL; it did not clear \(\beta\)-VLDL or apoE-HDL. These studies suggest that chylomicron uptake by bone marrow macrophages is more specific than uptake by the liver, which includes \(\beta\)-VLDL and apoE-HDL uptake as well. Because the clearance of \(\beta\)-VLDL and apoE-HDL from the plasma is mediated by apoE, bone marrow uptake does not appear to be specific for apoE-containing particles. The preferential uptake of triglyceride-rich lipoproteins by bone marrow macrophages is in agreement with the in vitro observation that macrophages can take up chylomicrons and VLDL obtained from hypertriglyceridemic patients (60, 61); it is also in agreement with in vivo observations demonstrating that lipid-filled macrophages appear in patients with types I, III, and V hyperlipoproteinemia (62) and familial lipoprotein lipase deficiency (63), who are known to accumulate chylomicrons, chylomicron remnants, and hypertriglyceridemic VLDL in their plasma.

The extent of chylomicron uptake by bone marrow macrophages and the liver has been shown to be modulated by several factors. As already mentioned, at low triglyceride dosages the liver cleared more of the injected chylomicrons, whereas at higher doses the bone marrow cleared a larger fraction of these lipoproteins. When chylomicron remnants were injected, a greater fraction of these lipoproteins was cleared by the liver. The increased affinity of remnants for the liver, compared with chylomicrons, is well established (50). Furthermore, the addition of apoE to chylomicrons did not affect bone marrow uptake; however, it resulted in an increased rate of clearance by the liver and thereby in a much shorter half-life for the chylomicrons in the plasma. The effect of apoE agrees with previous studies (15–17) that demonstrate that addition of apoE results in faster clearance from the plasma and increased uptake in the liver. The level of apoE available in the postprandial state may limit the rate of clearance of chylomicrons and chylomicron remnants from the plasma.

It remains to be determined whether the uptake of triglyceride-rich lipoproteins by the bone marrow is common in other species, and what its role is in bone marrow metabolism. For example, preliminary studies suggest that chylomicron

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uptake by rat bone marrow represents a very small percentage of the total clearance. Furthermore, studies performed in sheep and dogs indicate that the liver is the major site of uptake (9, 33). However, in the study by Melchior et al. (33), the liver uptake of chylomicron [3H]retinol was 66% of the injected dose at 2 h in the dog. At 20 min, the liver and plasma accounted only for approximately 50–60% of the injected dose, suggesting that other organs are involved in chylomicron clearance in the dog. These observed differences between species may be due to differences in the histology of the bone marrow. For example, in the rabbit the endothelial cells lining the bone marrow sinuses are not tight and the perisinusoidal macrophages protrude through the endothelial cells (59), whereas such protrusion does not occur in the bone marrow of rats. It will be important to obtain data on nonhuman primates and humans to determine the significance of bone marrow macrophages in chylomicron metabolism in these species.

The role of chylomicron uptake by the bone marrow may be related to the delivery of substrates (fatty acids and cholesterol) for blood cell formation, to the delivery of fat-soluble vitamins, or to the delivery of lipid for expansion of the triglyceride stores within the abundant adipocytes in the marrow.

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