Chylomicron Metabolism

CHYLOMICRON UPTAKE BY BONE MARROW IN DIFFERENT ANIMAL SPECIES*

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Previously it was shown in rabbits that 20–40% of the injected dose of chylomicrons was cleared from the plasma by perisinusoidal bone marrow macrophages. The present study was undertaken to determine whether the bone marrow of other species also cleared significant amounts of chylomicrons. Canine chylomicrons, labeled in vivo with [14C]cholesterol and [3H]retinol, were injected into marmosets (a small, New World primate), rats, guinea pigs, and dogs. Plasma clearance and tissue uptake of chylomicrons in these species were contrasted with results obtained in rabbits in parallel studies. The chylomicrons were cleared rapidly from the plasma in all animals; the plasma clearance of chylomicrons was faster in rats, guinea pigs, and dogs compared with their clearance from the plasma of rabbits and marmosets. The liver was a major site responsible for the uptake of these lipoproteins in all species. However, as in rabbits, the bone marrow of marmosets accounted for significant levels of chylomicron uptake. The uptake by the marmoset bone marrow ranged from one-fifth to one-half the levels seen in the liver. The marmoset bone marrow also took up chylomicron remnants. Perisinusoidal macrophages protruding through the endothelial cells into the marrow sinuses were responsible for the accumulation of the chylomicrons in the marmoset bone marrow, as determined by electron microscopy. In contrast to marmosets, chylomicron clearance by the bone marrow of rats, guinea pigs, and dogs was much less, and the spleen in rats and guinea pigs took up a large fraction of chylomicrons. The uptake of chylomicrons by the non-human primate (the marmoset), in association with the observation that triglyceride-rich lipoproteins accumulate in bone marrow macrophages in patients with type I, III, or V hyperlipoproteinemia, suggests that in humans the bone marrow may clear chylomicrons from the circulation. It is reasonable to speculate that chylomicrons have a role in the delivery of lipids to the bone marrow as a source of energy and for membrane biosynthesis or in the delivery of fat-soluble vitamins.

Chylomicrons play a major role in the transport of intestinally absorbed lipids. Their triglycerides are partially hydrolyzed during circulation as a result of the action of endothelial brush border lipoprotein lipase (1). The resulting remnants are cleared primarily by the liver through a process mediated by apolipoprotein E (2–6) and modulated by apolipoprotein C and phospholipids (3, 5, 7). In rabbits, in addition to the liver, the bone marrow has been shown to be involved in the clearance of chylomicrons and chylomicron remnants (8). Chylomicrons, chylomicron remnants, and intestinally absorbed cholesterol were taken up by the rabbit bone marrow, the lipoproteins were degraded, and the cholesterol was stored. The cells responsible for clearance by the bone marrow were identified as perisinusoidal macrophages (8). Furthermore, there is evidence that the bone marrow of rabbits takes up phospholipid liposomes and low density lipoproteins (9, 10).

The present study was undertaken to evaluate the involvement of the bone marrow in chylomicron metabolism in other species. The results show that the bone marrow not only of rabbits, but also of marmosets (a small, New World primate), is involved in chylomicron metabolism.

MATERIALS AND METHODS

Animals and Diets—Male New Zealand White rabbits (2.0–3.0 kg body weight, Animal West, Soquel, CA) were maintained on Purina Rabbit Chow 5315 (Purina Mills, St. Louis, MO) fed ad libitum. Marmosets were raised at the Gladstone Foundation Laboratories and fed canned marmoset diet (ZuFrem, Hills Pet Products, Topeka, KS), supplemented with multiple vitamins, fruits, and rice cereal. Both male and female marmoset monkeys, weighing 300–400 g, were used in this study. Adult mongrel dogs (University of California, San Francisco) were fed Purina Dog Chow. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA), weighing 200–400 g, were used in this study. Adult mongrel dogs (University of California, San Francisco) were fed Purina Dog Chow. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA), weighing 200–400 g, were used in this study. Adult mongrel dogs (University of California, San Francisco) were fed Purina Dog Chow. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA), weighing 200–400 g, were used in this study. Adult mongrel dogs (University of California, San Francisco) were fed Purina Dog Chow. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA), weighing 200–400 g, were used in this study.

For metabolic studies, animals were fasted overnight prior to metabolic studies.

Chylomicron and Chylomicron Remnant Preparation—Canine chylomicrons were obtained from a lymph fistula created at the left jugular vein essentially as described (11, 12). Mocha Mix (Presto Food Products, Industry, CA), cholesterol, and sucrose were fed to the dogs to induce chylomicron synthesis (8). Nascent chylomicrons were labeled in vivo with [14C]cholesterol and [3H]retinol as described previously (8). Chylomicrons were isolated from the lymph by ultracentrifugation (SW 28 rotor, 28,000 rpm, 90 min, 20 °C). In chylomicrons, more than 90% of the [3H]retinol and more than 70% of the [14C]cholesterol were esterified, suggesting that the particles were primarily derived from the intestine.

Chylomicron remnants were prepared by circulating chylomicrons in heptatectomized rabbits for 30 min. A functional hepatectomy was performed by ligating the superior and inferior mesenteric and celiac arteries, along with the portal vein (8). Ligatures were also placed around the base of the liver lobes. Femoral arteries were also tied to decrease the bone marrow uptake of chylomicrons. Chylomicron remnants were isolated from the plasma of heptatectomized rabbits by ultracentrifugation (SW 28 rotor, 28,000 rpm, 2 h 45 min, 4 °C) (8).

In Vivo Metabolic Studies—The in vivo metabolic studies were performed on anesthetized animals that had been fasted overnight.
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(15-18 h). The lipoproteins were injected into either a femoral, jugular, or ear vein. Blood samples were collected at designated time points from the contralateral femoral or ear artery or the jugular vein. Aliquots of plasma samples were counted for 10 min in a liquid scintillation counter (LS7500, Beckman Instruments). At the end of each experiment the animals were perfused at 80-90 mm of mercury pressure through the left ventricle. Rabbits were first perfused with about 500-600 ml of ice-cold minimal essential medium (GIBCO) and then with the same amount of ice-cold 3% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.3. Other animals were perfused similarly, except for the amount of the medium and glutaraldehyde solution used: 300-300 ml for marmosets and rats, 360-360 ml for guinea pigs, and 2500-3000 ml for dogs. The liver, bone marrow, spleen, kidney, lung, adrenals, heart, and adipose tissue were obtained at the end of the perfusion. Tissue slices (0.1-0.5 g) were digested in 0.5 ml of 6 N KOH, and [14C]cholesterol and [3H]retinol were extracted into hexane as described (8). The hexane extracts were evaporated, suspended in 0.5 ml of 100% ethanol, and counted in the presence of 10 ml of nonaqueous scintillation mixture (Beckman).

For electron microscopy, the spleen and the bone marrow from the femur were subjected to post-fixation, dehydration, embedding, and sectioning as described (8). The sections were examined using a JEOL electron microscope (model 100CXII).

Calculation of the percentage of injected dose of lipoproteins remaining in the plasma was based on the estimate that plasma volumes of rabbit, marmoset, dog, rat, and guinea pig constituted 3.5, 3.5, 4.35, 3.13, and 3.5% of body weight, respectively. The uptake of chylomicrons/g of tissue was obtained from duplicate determinations. In the case of bone marrow, two samples were obtained from both the femur and tibia and the uptake values were averaged. The organ uptake was based on the actual weight of the organs, except for the bone marrow. The bone marrow was estimated to be 2.2% of the total body weight in dogs and rabbits (13-16), whereas in rats it was estimated to be 3.0% (17), and in guinea pigs 1.75% (18). The bone marrow weight in marmosets was estimated to be 2.2% (see "Discussion").

Tissue Distribution of Intestinally Absorbed [14C]Cholesterol—The rabbits received 25 μCi of [14C]cholesterol in 0.5 ml of corn oil followed by 20 ml of Mocha Mix (8). Rats and guinea pigs were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ) and were given [14C]cholesterol (2.0-2.5 μCi) in 0.6 ml of corn oil via gastric intubation. All animals were euthanized 4.5 or 6 h after the injection of radiolabeled cholesterols. The tissues were collected, then digested in 6 N KOH, and lipids were extracted as described above and in Ref 8.

RESULTS

Chylomicron Clearance by the Bone Marrow in Various Animals

Canine thoracic duct chylomicrons labeled in vivo with [14C] cholesterol and [3H]retinol were injected into normal fasted rabbits, marmosets, rats, guinea pigs, and dogs. The plasma die-away and tissue uptake were determined. The metabolism of chylomicrons in rabbits served as the basis for comparison of the metabolism of these lipoproteins in other species. As summarized in Table I, the same chylomicron preparations were used in parallel in rabbits, marmosets, rats, guinea pigs, and dogs.

Rabbits—As established previously (8), chylomicrons were rapidly cleared from the plasma of rabbits and were taken up by both the liver and bone marrow. A typical plasma clearance and tissue distribution at 30 min after injection of the radiolabeled chylomicrons is shown in Fig. 1, A and B. Using four different preparations of chylomicrons (Table I), we determined that 23-35% of the injected dose of chylomicrons was retained in the plasma at 20-30 min after injection and that the uptake was primarily by the liver (15-48%) and bone marrow (14-35%). Likewise, chylomicron remnants were cleared by both the liver and the bone marrow, and in a hepatotomized rabbit the bone marrow was the principal site responsible for chylomicron catabolism (Table I).

Marmosets—The plasma clearance and tissue uptake of chylomicrons in marmosets were compared with results obtained in the rabbit. As shown in Fig. 1C, 37 and 29% of the injected dose of [14C]cholesterol- and [3H]retinol-labeled chylomicrons, respectively, remained in the plasma at 30 min. The liver accumulated 28% of the [14C]cholesterol and 25% of the [3H]retinol. The bone marrow clearance was responsible for the clearance of 13 and 9% of the [14C]cholesterol- and [3H]retinol-labeled chylomicrons in the injected dose, respectively (Fig. 1D), whereas the spleen accumulated 2-7% of the injected chylomicrons. Other organs studied contained no more than 1% of the injected lipoproteins (Fig. 1D).

As summarized in Table I, the amount of radiolabeled chylomicrons remaining in the plasma at 30 min ranged, in different experiments, from 29 to 46% of the injected dose, and the liver uptake varied from 23 to 28%. The bone marrow took up 6-13% of the injected chylomicrons, whereas the spleen had 2-7% of the injected chylomicrons. Furthermore, the chylomicron remnants were rapidly cleared from the plasma of marmosets (28 and 22% of the injected [14C]cholesterol-labeled remnants remained in the plasma at 20 and 60 min, respectively). At 60 min, 29 and 15% of the injected dose of [14C]cholesterol-labeled chylomicron remnants were within the liver and the bone marrow, respectively (Table I).

Even though the calculations of the uptake of chylomicrons by marmoset bone marrow were based on the assumption that the bone marrow constitutes 2.2% of the body weight (see "Discussion"), an estimate based on the values established for other animals (13-18), chylomicron uptake could be directly calculated as uptake/g of tissue rather than per organ. This uptake was then compared to the chylomicron uptake/g of tissue in the rabbit. As shown in Table II, the chylomicron clearance/g of bone marrow in both the rabbit and marmoset was similar (0.5-1.1% of the injected dose/g). Likewise, rabbit and marmoset chylomicron uptake/g of bone marrow was approximately equal to the uptake/g of liver. However, as shown in Fig. 1D, marmoset liver (4.6 ± 0.5% (n = 6)) cleared a larger fraction of chylomicrons compared to rabbit liver (2.8 ± 0.5% (n = 30)) because it constitutes a larger portion of the body weight. In contrast, the spleen of both animals takes up a large percentage of the injected dose/g (Table II); however, the spleen is a relatively small organ in marmosets (0.1 ± 0.03% of total body weight, n = 7) and rabbits (0.05 ± 0.01%, n = 30) and thus only accounts for 1-7% of the clearance of chylomicrons/organ.

Rats—Chylomicrons were cleared more rapidly from rat plasma (Fig. 1E) as compared with rabbits (Fig. 1A) and marmosets (Fig. 1C). At 30 min, only 5.0% of the [14C] cholesterol and 4.0% of the [3H]retinol of the injected dose were retained in the rat plasma. Liver uptake accounted for 84% of the [14C]cholesterol- and 76% of the [3H]retinol-labeled chylomicrons. The spleen had the next highest uptake, with 8% of the [14C]cholesterol and 5% of the [3H]retinol. In this experiment, the bone marrow accumulated 6% of the [14C]cholesterol and 2% of the [3H]retinol (Fig. 1F).

As summarized in Table I, chylomicron clearance from the plasma was rapid in all rats studied. The clearance by the liver ranged from 54 to 88% of the injected dose. In contrast, the uptake by the bone marrow ranged from less than 1% to about 6% of the injected dose. The uptake by the spleen ranged from 5 to 9%. These studies suggest that the liver is the major organ for chylomicron clearance in rats. However, the uptake of chylomicrons by the bone marrow increased when chylomicron clearance was studied in a hepatotomized rat. In a hepatotomized rat, 61% of the [14C]cholesterol- and 55% of the [3H]retinol-labeled chylomicrons remained in the plasma at 30 min, and the bone marrow accumulated 11% of the [14C]cholesterol and 10% of the [3H]retinol (Table I).
contrast to the rat, the bone marrow from a heptatectomized rabbit cleared 32% of the injected dose (Table I). These experiments suggest that rat bone marrow has the potential to take up significant quantities of chylomicrons.

Guinea Pigs—In a typical experiment in guinea pigs, 12% of the [14C]cholesterol and 6-15% of the [3H]retinol injected would be accounted for in the adipose tissue. Therefore, adipose tissue from the chest wall of guinea pigs accumulated 0.26-0.51% of the injected dose. In contrast, the perinephric adipose tissue of guinea pigs accumulated 0.02-0.03%, ~0.01%, and 0.09-0.15% of the injected dose/g of tissue, respectively. In the rat, the subcutaneous adipose tissue accounted for 0.01-0.02% of the injected dose. Assuming the fat content to be 7.1% of the body weight for guinea pigs (as has been reported in rats (19)), approximately 10-16% of the [14C]cholesterol and 6-15% of the [3H]retinol injected would be accounted for in the adipose tissue. Therefore, adipose tissue may be responsible for much of the chylomicron catabolism in guinea pigs.

Dogs—At 30 min, the dog retained 11% of the [14C]cholesterol- and 10% of the [3H]retinol-labeled chylomicrons in the plasma (Fig. 11 and Table I). At this time the liver had accumulated 63% of the [14C]cholesterol and 52% of the [3H]retinol, the spleen took up the next highest amount, clearing about 1% of the injected chylomicrons. The bone marrow uptake was 0.2-0.3% of the injected dose (Fig. 1J and Table I). Other tissues studied accumulated less than 0.4% (Fig. 1J). These experiments suggest that the liver is by far the organ with the largest role in chylomicron catabolism in dogs.
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FIG. 1. Representative plasma clearance and tissue uptake of canine chylomicrons in the rabbit, marmoset, rat, guinea pig, and dog. Overnight-fasted animals were anesthetized, and canine chylomicrons were injected into the femoral, jugular, or ear vein. Plasma samples were obtained at designated time points. Animals were perfusion fixed 30 min after injection of the chylomicrons, and tissue uptake was determined. Panels A and B represent plasma clearance and tissue uptake, respectively, in a rabbit. The rabbit received 150 mg of chylomicron triglyceride/kg of body weight. Panels C and D represent plasma clearance and tissue uptake, respectively, in a marmoset injected with 100 mg of triglyceride/kg of body weight. The plasma clearance and tissue uptake of chylomicrons in the rat are shown in panels E and F, respectively, whereas panels G and H represent plasma clearance and tissue uptake in the guinea pig. The rat and guinea pig received 200 mg of triglyceride/kg of body weight. The plasma clearance and tissue uptake in the dog are presented in panels I and J, respectively. The dog was injected with 150 mg of triglyceride/kg of body weight.

Tissue Uptake of Intestinal Lipoproteins Induced by Feeding Unsaturated Fat and [14C]Cholesterol

Consideration was given to the possibility that the uptake of chylomicrons by the spleen of rats and guinea pigs and the lack of uptake by the bone marrow in these two species were secondary to the use of canine lipoproteins. To rule out this possibility, [14C]cholesterol was administered along with unsaturated fat by gastric intubation to label endogenous intestinal lipoproteins. Rabbits were studied in parallel for comparison. The [14C]cholesterol was used in these studies because we had previously shown that it was retained within the bone marrow for several hours after uptake of the radiolabeled chylomicrons (8). Regardless of the potential complication that the [14C]cholesterol could be reutilized for endogenous lipoprotein production or transferred to other plasma lipoproteins, the tissue distribution of [14C]cholesterol should provide at least a qualitative estimate of the in vivo sites of chylomicron catabolism. In these studies, animals were euthanized at 4.5 or 6 h after the administration of the [14C]cholesterol, and the uptake of the radiolabeled lipoproteins determined per g of tissue. As shown in Table III, the various animals absorbed different quantities of the radiolabel; however, the data confirmed the observations obtained with injected canine chylomicrons. In rats and guinea pigs the liver took up a much larger fraction of the [14C]cholesterol-labeled intestinal particles/g of tissue (Table III). As shown in Table III, the ratio of liver to bone marrow uptake was at least 2-
Perisinusoidal macrophages in rabbit bone marrow protrude through the endothelium and have access to the marrow sinuses. A very similar electron microscopic image was observed within the bone marrow of marmosets. As shown in Fig. 2A, chylomicron size particles accumulated within perisinusoidal macrophages of the marmoset bone marrow. As in the rabbit, the bone marrow of marmosets displayed macrophages that extended through the endothelium into the sinuses (Fig. 2B) and accumulated chylomicron size lipoproteins. These observations are consistent with the biochemical data demonstrating that the bone marrow of marmosets and rabbits represents a major site for chylomicron catabolism.

The sites of chylomicron uptake by the spleen of the various animals were determined. Despite the fact that the spleen of rabbits and marmosets accounted for only low levels of uptake of chylomicrons, chylomicron size particles could be identified within splenic macrophages in rabbits and marmosets (Fig. 3, A and B), guinea pigs, and dogs (data not shown). Likewise, chylomicron size lipoproteins were abundant within macrophages (Fig. 3C) and entrapped within the interstitial spaces (Fig. 3D) of the rat spleen. It was not possible to determine the relative percentage of particles within the cells and trapped between cells. These results are consistent with the biochemical observation demonstrating that chylomicrons are taken up by the spleen.

### DISCUSSION

In a previous study (8), rabbit and canine chylomicrons were shown to be cleared almost entirely by the liver and bone marrow of rabbits. In the present study, canine thoracic duct chylomicrons, labeled in vivo with [14C]cholesterol and [3H]retinol, were used to study uptake by the bone marrow in the marmoset, rat, guinea pig, and dog. Approximately two-thirds of the injected chylomicrons were cleared from the circulation of these animals within 30 min after intravenous injection. This rapid clearance of chylomicrons is consistent with results from previous studies reported for rats, rabbits, and dogs (12, 19–29). In dogs, rats, and guinea pigs, the chylomicrons cleared from the plasma were recovered primarily in the liver. The observation that the liver is the primary organ responsible for chylomicron clearance is consistent with other studies in dogs and rats (12, 19, 21, 25–29). However, in rabbits and marmosets the bone marrow also cleared a significant fraction of chylomicrons, whereas in rats, guinea pigs, and dogs the bone marrow was less active.

The calculation of the uptake of chylomicrons by bone marrow is based on the total organ weight of the marrow. This value has been very carefully and exhaustively determined in several species but not in marmosets or other non-human primates. In rabbits, bone marrow represents 2.2–2.5% of the body weight (13–15), whereas it ranges from 1.9 to 2.4% in dogs (16), 3.0% in rats (17), and 1.75% in guinea pigs (18). We have used 2.2% as the factor for conversion of data from values/g of organ to values/organ in the marmoset. This estimation was based on the following reasons. Marmosets have a total body weight approximately equal to that of rats and guinea pigs, and as noted, bone marrow in these two animals averages 1.75–3.0% of the body weight. Furthermore, based on values obtained per g of bone marrow in rabbits and marmosets (Table II), both the rabbit and marmoset marrow take up very similar amounts of radiolabeled chylomicrons. In addition, the uptake of [14C]cholesterol and [3H]retinol by bone marrow was similar to that of the liver (per g) in these animals. Finally, in rabbits, the bone marrow weight is equal to approximately two-thirds of the weight of the liver (15). If this ratio is similar in the marmoset, the actual bone marrow...
weight would be at least 3% of the body weight. For all of these reasons we have used 2.2% as a reasonable estimate.

Macrophages were responsible for the uptake of chylomicrons in the bone marrow of rabbits and marmosets. Specifically, the uptake was restricted primarily to perisinusoidal macrophages, which had access to the circulating blood through processes extending into the marrow sinuses. However, in some other organs, macrophages have access to the plasma but do not take up chylomicrons (e.g., the Kupffer cells in the rabbit liver do not (8)). Furthermore, even though rabbit bone marrow macrophages have access to all circulating lipoproteins, only triglyceride-rich lipoproteins are taken up (8). Previously it has been reported that human bone marrow accumulates large quantities of triglyceride-rich lipoproteins present in the plasma of patients with type I, III, or V hyperlipoproteinemia (30).

In contrast to rabbits and marmosets, the bone marrow of dogs and guinea pigs does not appear to be involved to a significant extent in the clearance of chylomicrons (less than 2% of the injected dose). It is possible that the bone marrow of these species lacks the ability to take up chylomicrons because the bone marrow macrophages in these species do not have easy access to large lipoprotein particles in the blood sinuses. Alternatively, in these species chylomicrons may not be required in the bone marrow because other lipoproteins or transport molecules fulfill the same functions as chylomicrons. In addition, it is possible that the bone marrow of the rabbit and marmoset performs a function requiring chylomicrons and chylomicron remnants that is served by another organ in guinea pigs and dogs, e.g., the spleen.

Rat bone marrow appears to take up chylomicrons at a level below that of rabbit and marmoset marrow and above that of guinea pig and dog marrow. Although the liver cleared the highest amounts of chylomicrons in the rat, the bone marrow did take up 1–6% of the injected dose. Small amounts of chylomicrons have been shown to be cleared by the rat bone marrow (27). Furthermore, in the hepatectomized rat, the chylomicron uptake by bone marrow increased to 10–11% of the injected dose. Previous studies have demonstrated that chylomicrons are cleared from the plasma of hepatectomized rats, rabbits, and dogs (8, 21, 29, 31, 32). In the rabbit, the uptake of chylomicrons by bone marrow was almost quantitative (Table I and Ref. 8). The rat bone marrow does have at least a limited ability to take up chylomicrons in a hepatectomized animal. In high concentrations and with the long circulation time that occurs in hepatectomized rats, the chylomicrons may cross the bone marrow endothelium and reach the macrophages.

Several caveats concerning these data should be mentioned. First of all, in many of our studies we have injected high levels of chylomicrons or fed a fat-rich diet to induce a significant concentration of intestinal lipoproteins. Even though these levels may seem large, one needs to recall that humans absorb very large quantities of lipids (several grams) after a typical meal. However, in other studies we have attempted to administer much lower levels of chylomicrons, to infuse whole lymph slowly, or to feed only the low-fat animal chow containing the radiolabeled cholesterol (8). In all of these studies with rabbits, the bone marrow took up significant quantities of chylomicrons or intestinally absorbed lipids. Second, even though all of our data are consistent with chylomicrons and chylomicron remnants being principally involved in the delivery of the radiolabeled cholesterol and retinol to the bone marrow, we cannot entirely rule out the possibility that some of the cholesterol might be transferred to other lipoproteins (especially in the rabbit and marmoset, which have cholesteryl ester transfer protein) or reutilized in the synthesis of endogenous lipoproteins and be taken up by the bone marrow. Studies are in progress using an isolated hind-limb perfusion system for direct measurement of bone marrow uptake of lipoproteins.

The physiological importance of the uptake of chylomicrons by the bone marrow is not known. However, there are several possibilities. First, chylomicrons and chylomicron remnants may serve to deliver lipids or fat-soluble vitamins to the bone marrow. Fatty acids may serve as an energy source and cholesterol may be used in membrane biosynthesis during hematopoiesis. Fat-soluble vitamins are transported by lipoproteins, specifically chylomicrons, which may deliver these vitamins to the marrow. For example, vitamin E is known to play a key role in stabilizing red blood cell membranes. In fact, autohemolysis can be corrected in some patients by administering vitamin E (33). Furthermore, it has been suggested that large oral doses of vitamin A can be used to maintain remission in children with acute myeloid leukemia (34). The possible importance of chylomicrons in this process
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FIG. 3. Electron micrographs of the spleen of different species 30 min after the injection of chylomicrons. A, the spleen of a rabbit given an injection of 150 mg of chylomicron triglyceride/kg of body weight has macrophages that contain chylomicron size particles. ×3450. B, the spleen of a marmoset injected with 100 mg of chylomicron triglyceride/kg of body weight has macrophages that contain intracellular chylomicron size particles and lipid droplets (LD). ×3450. C and D, the spleen of a rat injected with 33 mg of chylomicron triglyceride/kg of body weight contains intracellular chylomicron size particles within a macrophage (panel C) and trapped in the intracellular space between cells (panel D). ×3450.

was suggested in in vitro studies in which vitamin A delivered to promyelocytic leukemic cells on chylomicrons was the most effective means of inducing differentiation and inhibiting proliferation of these cells (35).

A second possible role for chylomicron uptake by the bone marrow is to maintain adipocyte lipid stores. The turnover of fatty acids in bone marrow adipocytes of rabbits is five times higher than in extramedullary adipocytes (36); thus, the adipocytes appear to play a dynamic role in the bone marrow. Furthermore, there is an inverse relationship between high hematopoietic cell synthetic activity and adipocyte triglyceride content (37). The triglyceride may be mobilized as an energy source or may simply provide space for expansion of the hematopoietic elements. In addition, adipocytes may be involved in regulating the hematopoietic microenvironment of the bone marrow. In vitro hematopoietic cultures appear to require marrow stromal elements, including adipocytes, for the normal maintenance, proliferation, differentiation, and maturation of bone marrow stem cells (38). Further studies are required to distinguish among these possibilities.

In rats, guinea pigs, and dogs, the spleen was the organ with the second highest uptake of radiolabeled chylomicrons (after the liver). However, the spleen only accounted for ~1.5% of the injected dose of chylomicrons in dogs and 5–13% of the injected dose in rats and guinea pigs, calculated per organ (Table I). Previously, the uptake of chylomicrons has been shown to occur in the rat spleen (27, 28). To establish that the uptake by rat and guinea pig spleen was not due to the use of canine chylomicrons or to chylomicron alteration during isolation, these animals were fed [14C]cholesterol plus fat to label endogenous intestinal particles. Significant amounts of radiolabeled lipoproteins were detected in the spleens of these animals (Table III). In fact, the liver and the spleen in rats and guinea pigs accumulated similar amounts of radiolabel when calculated per g of tissue. This demonstrates that the spleen does accumulate native endogenous intestinal lipoproteins. In addition, it was noted that a similar amount of [14C]cholesterol was also taken up by the rabbit spleen (liver/spleen ratio; Table III). However, because the spleens of rats and guinea pigs are much larger than those of rabbits, there is a greater total uptake (per organ) in the spleens of rats and guinea pigs. It is intriguing to consider
that the relative differences in the uptake of chylomicrons by the bone marrow versus the spleen (greater bone marrow uptake in rabbits and marmosets versus greater spleen uptake in rats and guinea pigs) might reflect different metabolic roles for these organs in the different species. Furthermore, the difference between bone marrow and spleen uptake of chylomicrons may provide insights into the role of chylomicron catabolism in these organs, both of which function in hematopoiesis. A correlation between hematopoiesis in the bone marrow and the spleen and uptake of chylomicrons could be of significant physiological importance.

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