Uptake and processing of remnants of chylomicrons and very low density lipoproteins by rat liver

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Abstract In the rat, chylomicron remnants and very low density lipoprotein (VLDL) remnants are taken up into the liver by high affinity processes and appear to undergo degradation by lysosomes. The relationship of this catabolic process to the known pathways of uptake and degradation of low density lipoproteins (LDL) and the involvement of nonparenchymal cells are addressed in these studies. We have utilized both light and electron microscopic radioautography to determine whether the pathway of intracellular transport and catabolism resembles that established for LDL in hepatocytes. Radioiodinated plasma VLDL remnants and lymph chylomicron remnants were injected into femoral veins of rats and the livers were fixed by perfusion 3 to 30 minutes later. Quantitative light microscopic radioautography showed little or no accumulation of grains over Kupffer cells. Electromicroscopic radioautography confirmed these observations and, in addition, demonstrated that very few grains were associated with endothelial cells. The processing of the remnant particles closely resembled that of LDL. Following an initial association of grains with the parenchymal cell plasma membrane, frequently in regions in close proximity to clathrin-coated endocytic pits, the grains were found in endocytic vesicles just beneath the plasma membrane. By 15 minutes the grains were found over multivesicular bodies located in the Golgi-lysosome region of the cell. Thirty minutes after injection, radioautographic grains began to be associated with secondary lysosomes. These data indicate no significant role for nonparenchymal cells in the internalization and subsequent degradation of triglyceride-rich lipoproteins, and provide evidence that the processing of remnants as well as LDL follows the classical pathway of receptor-mediated endocytosis.—**Jones, A. L., G. T. Hradek, C. Hornick, G. Renaud, E. E. T. Windler, and R. J. Havel.** Uptake and processing of remnants of chylomicrons and very low density lipoproteins by rat liver. *J. Lipid Res.* 1984. *25:* 1151–1158.

Supplementary key works hepatocytes • electron microscopic radioautography • lysosomes • multivesicular bodies • coated pits • vesicles • receptor-mediated endocytosis

It is now well accepted that the terminal steps in the metabolism of circulating chylomicrons take place in the liver (1–3). To a variable extent among mammals, this seems to be true for very low density lipoproteins (VLDL) as well (4, 5). The evidence from several animal models suggests that, following the interaction of these triglyceride-rich lipoproteins with lipoprotein lipase within extrahepatic blood capillaries (6), the resulting remnants are rapidly bound to high affinity receptors on liver cells (7). In the rat, low density lipoproteins (LDL) are bound to specific receptors on hepatic parenchymal cells, internalized by a well-defined pathway and catabolized in lysosomes (8–10). Chylomicron remnants (11) and VLDL remnants (4, 12) are also thought to be processed by lysosomal pathways, but the relationship of these pathways to that described for LDL has not been defined in detail, and the role of nonparenchymal (Kupffer and endothelial) cells in remnant uptake and processing is controversial (4, 11, 13–16). In the current studies, we have used methods of light and electron microscopic radioautography applied previously to the study of the LDL pathway in rat liver (10) to address these questions.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 250–300 g and fed standard Purina Chow for at least 1 week were anesthetized with diethyl ether. Their portal veins were exposed and cannulated with a 26-gauge needle attached to a 1-ml syringe containing the lipoproteins to be injected. The lipoproteins were injected over a period of 1 min.

Preparation of lipoproteins

Hepatic VLDL and small lymph chylomicrons were prepared as described (17) and radioiodinated with ^{125}I

Abbreviations: LDL, low density lipoproteins, VLDL, very low density lipoproteins; MVB, multivesicular bodies.

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by the iodine monochloride method (8). Remnants were obtained by aortic puncture 30 min after injection of labeled VLDL or chylomicrons (~1.4 mg of protein) into the femoral vein of functionally eviscerated rats (8). Chylomicron and VLDL remnants were separated by centrifugation of the serum for 10^8 g_{av}-min, after adjusting the serum to a density of 1.019 g/ml with D_2O and layering it beneath Krebs-Henseleit buffer (17).

Uptake of ^{125}I chylomicron or VLDL remnants

After femoral injection of either chylomicron remnants or VLDL remnants (0.5–0.9 mg of protein; 60–120 × 10^6 cpm ^{125}I) into five male Sprague–Dawley rats, the livers were fixed 3, 15, or 30 min later (for chylomicrons) and 20 min later (for VLDL). The fixative (2.5% glutaraldehyde and 0.8% paraformaldehyde in 0.2 M $NaHCO_3$, pH 7.3) was infused directly into the portal vein for 2 min (18). After perfusion-fixation the tissue was cut into 1 × 1 mm blocks and immersed in the same fixative for an additional 2 hr. The fixed tissue was then washed overnight in 0.2 M $NaHCO_3$, pH 7.4, at 4°C and osmicated in 1% osmium tetroxide containing 1.57% KCN for 90 min. It was then washed in 0.2 M $NaHCO_3$ for 10 min, dehydrated in ethanol, and embedded in Epon. In order to determine uptake of ^{125}I, ten random pieces of tissue were removed from the liver after the glutaraldehyde/paraformaldehyde perfusion-fixation and assayed in a gamma spectrometer.

Radioautography

Thick sections (0.5 mm) and thin sections (900 Å) were cut for light and electron microscopic radioautography, respectively. The thick sections were placed on glass slides and coated with Kodak NTB-3 emulsion and developed 2 weeks later in Kodak D-19 developer. Thin sections were placed on parlodion-coated grids, overlaid with a monolayer of Ilford L-4 emulsion, exposed for 6 weeks, developed in Kodak D-19, and stained with lead citrate.

The resolution half-distance (19) for proteins labeled with ^{125}I in our laboratory was found to be 850 Å. Grains were quantified by means of a circle analysis (20). An overlay of a 2 half-distance circle was placed over the radioautographic grains seen in the electron micrographs such that the center of the circle was located over the approximate center of the grain. Organelle classes falling within the circle were given full or partial credit if two or three organelles were found within the circle. The percentage of grains associated with each class is reported.

For each liver, approximately 100 electron micrographs containing 150–160 radioautographic grains were photographed in random grid squares. Within a given grid square, every grain was photographed at a constant magnification. Midlobular areas were utilized for these studies.

RESULTS

Light microscopic radioautography

Qualitative light microscopic radioautography was performed on thick sections from all livers, which contained 7–23% of the injected ^{125}I. Approximately 25% of the ^{125}I was lost during fixation, washing, and osmication, and an additional 35% was lost during dehydration and embedding of the tissue. Because our tissue processing method is known to result in marked lipid loss, the remaining ^{125}I represents almost entirely ^{125}I bound to protein (4). For chylomicron remnants, the largest concentration of grains was seen 15 min after injection (**Fig. 1**). There was never a notable accumulation of grains over the Kupffer cells but an association of grains with endothelial cells could not be excluded because the limits of resolution of radioautography at the light microscopic level do not permit differentiation between grains associated with the plasma membranes of parenchymal cells and with endothelial cells.

Electron microscopic radioautography

The two most fundamental observations made in this study were the remarkable predominance of radioautographic grains representing ^{125}I associated with lipoprotein remnants within liver parenchymal cells and not sinusoidal lining cells (**Table 1**), and the striking similarity of the processing of labeled remnants to that of LDL in normal and ethinyl estradiol-treated rats (10). Three minutes after injection of chylomicron remnants, one-half of the grains were on the plasma membrane of parenchymal cells (**Fig. 2a**). However, even at this early time, significant numbers of grains could already be observed over vesicles and multivesicular bodies (MVB) in these cells (Table 1). Numerous cell surface grains were within two half-distances from endocytic pits which appeared to contain clathrin coats. Although after 15 min numerous grains were still associated with the cell surface, many were over larger vesicles near the cell surface (Fig. 2b).

Also, at this time, as the fraction of grains associated with the surface fell, there was an increased concentration of grains over MVB whose matrix varied from low to high electron density (Fig. 2c). Most of the MVB were in the Golgi-lysosome region. In this location MVB and their contents (probably remnants) were clearly distinguishable from secretory vesicles of the Golgi complex that contained nascent VLDL (Fig. 2c and d). Essentially

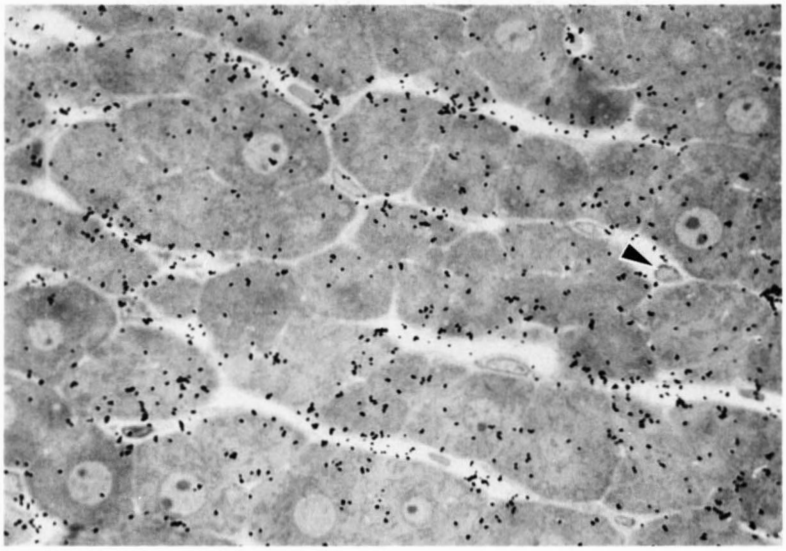

Fig. 1. Light microscopic radioautogram 15 min after the femoral injection of radioiodinated chylomicron remnants. Silver grains can be seen both at the surface and within the parenchymal cells. There is no evidence of grain accumulation over Kupffer cells (arrow). 500×.

no grains were associated with Golgi secretory vesicles and very few were associated with other components of the Golgi complex (Table 1). Thirty minutes after injection, the concentration of radioautographic grains associated with the plasma membrane of parenchymal cells was reduced to less than one-half that seen after 3 min, whereas grains associated with MVB had increased almost threefold. Initially, no grains were associated with secondary lysosomes but after 30 min almost 7% were associated with this structure (Table 1 and Fig. 2d). To determine the universality of remnant processing by liver, quantitative electron microscopic radioautography was performed on the liver from an animal 20 min after injection of VLDL remnants. The distribution of grains resembled that found for chylomicron remnants after 15 and 30 min (Table 1 and **Fig. 3**).

The only other significant localization of silver grains was in the smooth endoplasmic reticulum. This organelle occupies about 7% of hepatocyte volume and 20–30% of grains were associated with it under each experimental

TABLE 1. Distribution of radioautographic grains over liver cells and organelles after intravenous injection of lymph chylomicron remnants or plasma VLDL remnants

	Percent of Grains Scored				
	Chylomicron Remnants[a]				VLDL Remnants
	3 (175)[b]	15 (258)	15 (315)	30 (618)	20 (152)
Hepatocyte					
Plasma membrane	49.7	27.9	30.5	21.4	17.8
Small vesicle	8.0	10.5	5.4	3.4	3.3
Multivesicular body	8.0	10.8	14.0	22.3	33.0
Lysosome	0.0	1.6	0.3	6.8	2.6
Smooth endoplasmic reticulum	18.9	26.7	24.1	24.6	28.3
Rough endoplasmic reticulum	2.9	2.7	5.1	5.5	5.3
Golgi apparatus	1.1	0.7	5.1	4.0	2.0
Mitochondria	3.4	5.8	5.1	6.8	6.6
Other	2.0	3.3	2.5	1.2	0.3
Endothelial and Kupffer cell	6.0	10.0	8.0	4.0	1.0

[a] The first two columns represent results obtained with one preparation of chylomicron remnants; the second two columns represent results obtained with a separate preparation.
[b] First number denotes minutes after injection of ^{125}I-labeled chylomicrons or VLDL. Number in parentheses is the total number of grains scored.

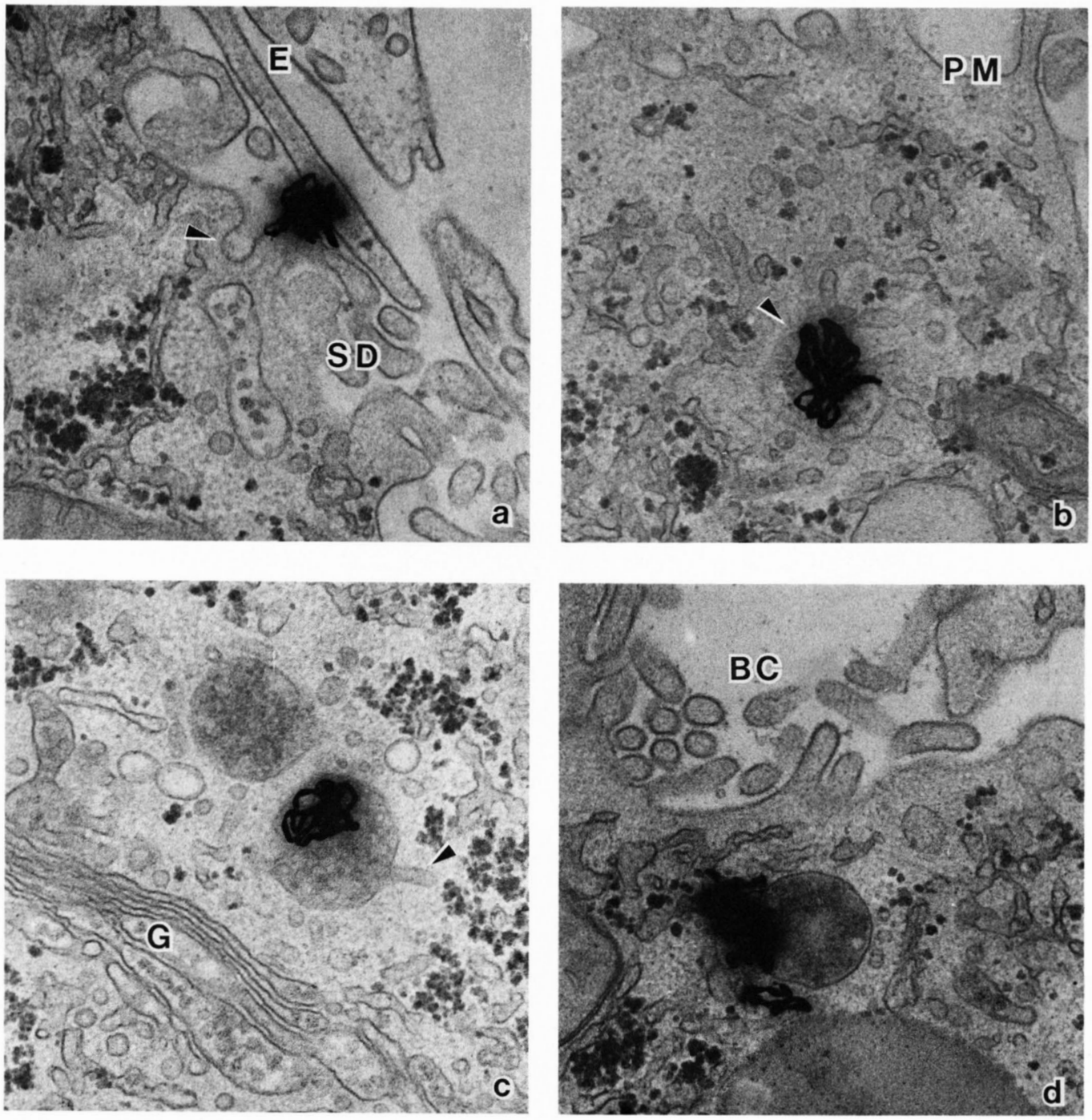

Fig. 2. Electron micrographs showing examples of the distribution of radio-labeled chylomicron remnant silver grains. 45,000×. 2a: Three min after injection of chylomicron remnants, approximately 50% of the grains are observed within two half-distances of coated pits (arrow). E, endothelial cell; SD, space of Disse. 2b: By 15 min many of the grains are located in large endocytic vesicles near the plasma membrane (arrow). PM, plasma membrane. 2c: At 15 min, grains are also associated with multivesicular bodies containing tail-like appendages (arrow) that are located in the Golgi-lysosome region of the cell. G, Golgi complex. 2d: By 30 min a substantial number of grains remains associated with multivesicular bodies, but 7% of the grains now are associated with secondary lysosomes. BC, bile canaliculi.

condition and at each time examined. A similar percentage of grains was associated with the smooth endoplasmic reticulum in our radioautographic studies of the hepatic uptake of LDL in normal and ethinyl estradiol-treated rats (10, 21).

DISCUSSION

Recent studies provide strong evidence that chylomicron remnants are processed in liver by a mechanism distinct from that for LDL (22–24). In homozygous

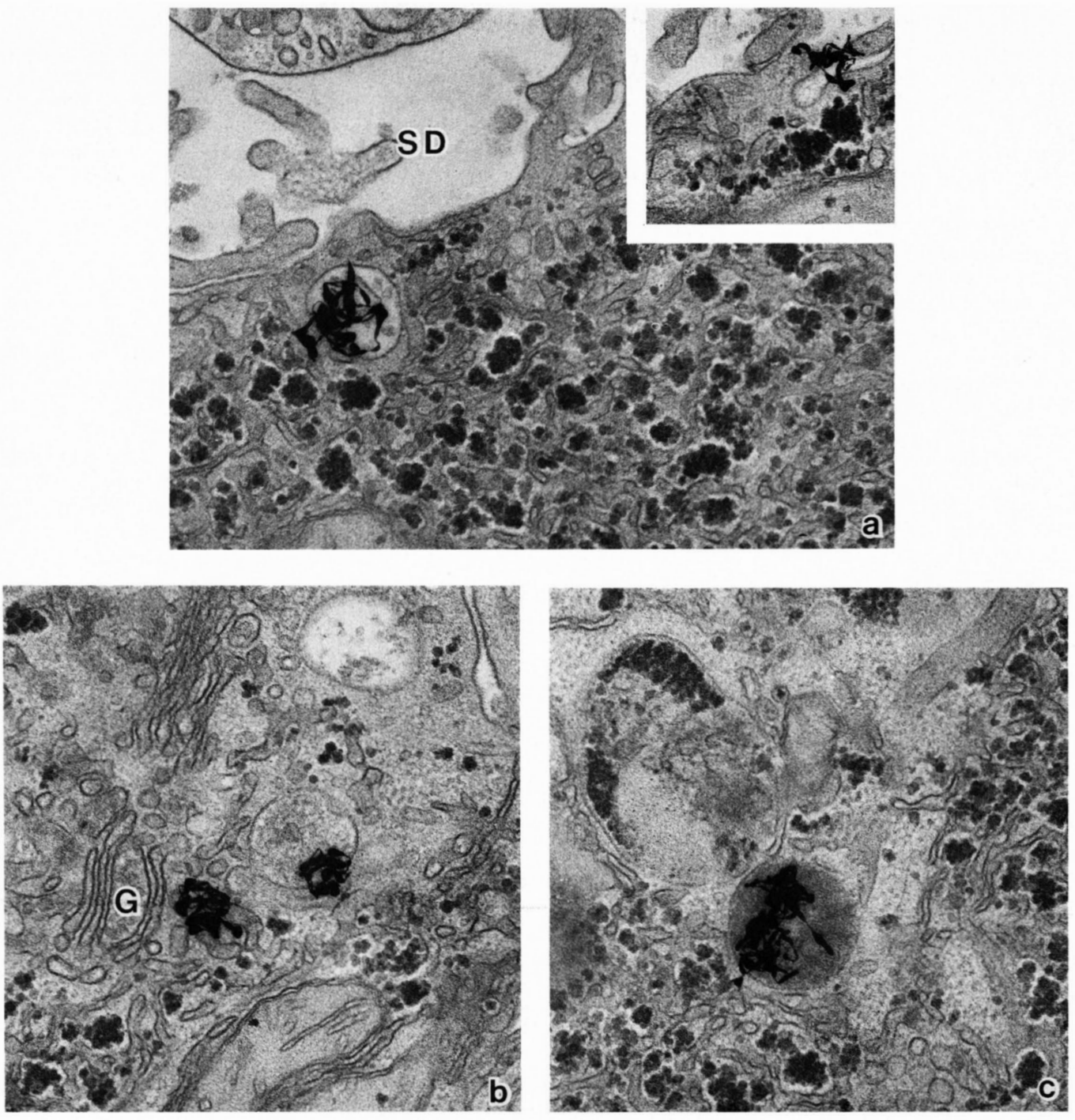

Fig. 3. VLDL remnant distribution in hepatocytes 20 min after injection. The distribution of grains is similar to that found for chylomicron remnants after 15 and 30 min. Fig. 3a shows grains near a coated pit (inset) and within larger vesicles just beneath the plasma membrane. Grains are also seen associated with multivesicular bodies (3b) and secondary lysosomes (3c). SD, space of Disse; G, Golgi complex. 40,000×.

Watanabe heritable hyperlipidemic (WHHL) rabbits, which have very low levels of functional LDL receptors in liver, chylomicron metabolism is unaltered (22), whereas that of LDL is inefficient and proceeds almost entirely by a pathway independent of the LDL receptor (7). In the current study, we demonstrate that all the steps in the hepatic processing of remnants derived from lymph chylomicrons, as deduced from electron microscropic radioautography, are indistinguishable from those that we have identified previously for LDL in livers of normal and estradiol-treated rats (10). *1*) The initial binding to the surface of the hepatic parenchymal

cell is partially, but by no means exclusively, in close proximity to coated pits. *2*) Initial binding is followed closely by the appearance of radioautographic grains in vesicular structures, larger than coated pits or coated vesicles, just beneath the plasma membrane of parenchymal cells. *3*) Within a few minutes grains become concentrated in MVB, often characterized by a tubular appendage and located predominantly in the bile canalicular pole of hepatocytes, in close proximity to the Golgi apparatus. *4*) Grains begin to concentrate in secondary lysosomes 20 to 30 min after injection of the lipoprotein in vivo. These results strongly support the concept that chylomicron remnants are taken up and processed by hepatic parenchymal cells by receptor-mediated endocytosis. Although the receptors for chylomicron remnants and LDL appear to be distinct, they evidently lead to the same pathway of internalization and degradation.

In the rat, the hepatic uptake of chylomicron remnants is mediated at least in part by apolipoprotein E (7). Although the LDL receptor binds lipoproteins containing apoE as well as apoB-100, results of binding studies with membranes prepared from dog liver suggest the presence of an apoE-binding site distinct from the LDL receptor (23). By contrast, no such site has been detected in similar membrane preparations of livers from WHHL homozygotes (22, 24). Recent studies in WHHL homozygotes indicate that the hepatic processing of VLDL remnants is mediated by the LDL receptor (25). It may be that apoB-48 (present in rabbit chylomicrons but not in VLDL (26)) participates in chylomicron remnant binding; alternatively, apoB-100 in VLDL remnants may prevent such binding. In the case of rat VLDL, binding may involve both receptors because hepatogenous VLDL contains a protein resembling apoB-48 as well as apoB-100 (27). In our experiments with labeled chylomicron remnants, a portion of the ^{125}I may have become associated with VLDL because some of the labeled proteins could have exchanged with the unlabeled protein of the VLDL in the plasma of recipient rats. We attempted to minimize such transfers by injecting large amounts of labeled chylomicrons into the functionally eviscerated rats (the injected protein was about twice that contained in the recipient rats' VLDL). We also injected a large amount of lipoprotein-protein into the recipients of the remnants. A close similarity of the distribution of radioautographic grains of chylomicron remnants and VLDL remnants to those obtained previously with LDL (10) would therefore not be expected if distinct intracellular pathways of catabolism were dictated by the different cell surface receptors.

The current studies also strengthen the evidence that MVB are an intermediate compartment in the processing of lipoproteins in rat liver (10, 21). The participation of MVB in LDL processing by normal liver was strongly indicated in our previous work, even though hepatic uptake of LDL was limited (10). The particles seen within MVB probably represent mainly remnant VLDL and chylomicrons. This interpretation is supported by observations on the composition of lipoproteins obtained from MVB isolated from livers of estradiol-treated rats (Hornick, C. A., R. L. Hamilton, E. Spaziani, G. H. Enders, and R. J. Havel, unpublished data).

Finally, the current studies support earlier findings of the predominant localization of cholesteryl ester-labeled (11) and cholesteryl ether-labeled chylomicrons (28) and radioiodinated VLDL (4) in hepatic parenchymal cells of the rat. Our results show a paucity of association of ^{125}I with either Kupffer or endothelial cells at any time up to 30 min after injection of labeled remnants of lymph chylomicrons or hepatic VLDL. In other studies, in which parenchymal and nonparenchymal cells have been separated from rat livers after injection of various labeled lipoproteins, inconsistent results have been obtained (4, 11, 13–16, 29, 30). Van Berkel and his associates have invariably found preferential association of radioiodine with nonparenchymal cells after injection of chylomicron remnants, VLDL remnants, LDL, or high density lipoproteins into normal rats (14, 15, 31). They have obtained similar results when nonparenchymal cells have been obtained after digestion of livers with pronase (which selectively destroys parenchymal cells) or after digestion with collagenase followed by separation of parenchymal and nonparenchymal cells by differential centrifugation. The latter method yields partially purified nonparenchymal cells, contaminated with partially destroyed remnants of parenchymal cells (blebs) (32). By contrast, others who have used differential centrifugation to separate the cells have found sucrose-labeled LDL to be taken up almost exclusively by parenchymal cells in both normal and WHHL rabbits (30). Isolated nonparenchymal cells also bind lipoproteins specifically more than parenchymal cells (33), but such binding is not followed by appreciable apparent internalization or degradation and its significance for lipoprotein metabolism in vivo remains uncertain.

In contrast to the uniform findings of substantial uptake of lipoproteins into nonparenchymal cells of normal rats, Harkes and Van Berkel (31) found that LDL taken up by livers of estradiol-treated rats is found mainly in parenchymal cells separated by differential centrifugation after collagenase digestion. This could reflect a greater extent of internalization of lipoproteins in the livers of the hormone-treated animals, making less available for dissociation from parenchymal cells during processing. Such dissociated lipoproteins might then be taken up by scavenger mechanisms into nonparenchymal cells. Our studies provide no evidence for

association of remnant lipoproteins with the surfaces of Kupffer or endothelial cells or for endocytosis of remnants by these cells. Although these observations do not exclude a role for nonparenchymal cells in the processing of remnant lipoproteins, they provide strong evidence that parenchymal cells are the site of virtually all internalization of remnants that leads to lysosomal catabolism.

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