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Degradation of Particulate Lipid by the Large-Granule Fraction of Rat Liver (38555)

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The physiologic role of the reticuloendothelial system (RES) in host defense involves both the phagocytic uptake and subsequent intracellular degradation of potentially harmful blood-borne particulate material (1). While depressed phagocytosis due to humoral or cellular defects has been previously noted (2, 3), altered macrophage function may also allow toxic macromolecules such as bacterial endotoxin, to be phagocytized but not readily degraded (4). In view of the dual importance of both phagocytosis and intracellular degradation in host defense, a technique employing a stable lipid emulsion (5) to evaluate both of these critical RES functions (phagocytosis and degradation) in the intact animal was developed (6, 7). Previous studies with this test particle indicated that most of the radiolabeled lipid test substance was localized and degraded within hepatic macrophages, i.e., Kupffer cells (6, 7). In an effort to characterize this intracellular digestive system, the capacity of different hepatic sub-cellular fractions to degrade the ^{131}I-triglyceride particles *in vitro* was evaluated. Furthermore, since lysosomes are plentiful within macrophages and possess the necessary hydrolytic enzymes (8, 9), specific attention was focused on lysosomal rich large-granule fraction (LGF) of the liver.

Materials and Methods. Experimental animals. Male rats of the Sprague–Dawley strain (Holtzman Co., Madison, WI.) weighing 280–320 g were maintained on Rockland Lab-Tek chow and tap water *ad libitum*. Animals were anesthetized lightly with ether prior to lipid injection or stunned by cervical dislocation before rapid liver removal for homogenization.

Preparation and administration of lipid emulsion. Radioiodinated "RE-test-lipid emulsion" (6, 7) was used to evaluate hepatic phagocytic and degradative activity. The anhydrous lipid base consisted of glycerol, ^{131}I-triolein (Mallinckrodt Nuclear, St. Louis, MO), and soya lecithin mixed in ratio of 10:10:1 by weight, respectively (5). Prior to use, the lipid base with a specific activity of 10 μCi/g was suspended in sterile 5% dextrose and water and incubated at 37° for 20 min. A 10% lipid emulsion was injected via the dorsal vein of the penis at a dose of 30 mg of base/100 g of body weight. For *in vitro* studies a dose of 1000 μg of base (480 μg of ^{121}I-triglyceride) prepared as a 1% emulsion was utilized.

Vascular clearance and hepatic localization. The initial disappearance of lipid particles from the circulation was evaluated by plotting the blood particle ^{131}I concentration semilogarithmically against time in minutes. Blood ^{131}I radioactivity was determined by radioassay of heparinized blood aliquots collected every 2 min for the first 10 min. Hepatic localization of the lipid was determined by radioassay of liver samples obtained from rats sacrificed at 15, 30, and 60 min postinjection. Localization was expressed as mg of ^{131}I-triglyceride/g of liver and the percent of the injected dose localized per total organ.

Preparation of hepatic fractions. A 10% homogenate of normal rat liver was prepared in cold 0.25 *M* sucrose using a Teflon pestle-glass tube homogenizer (Kontes Co., Vineland, NJ) driven at 920 rpm. Hepatic subcellular fractions were subsequently prepared according to the method of Weissmann and Thomas (10) by first centrifuging the 10% homogenate at 3300 g for 10 min to remove nuclei and cellular fragments and then centrifuging that supernatant at 10,000*g* for 25 min. The 10,000*g* sediment of large-granule fraction (LGF) was resuspended in 0.25 *M* sucrose to 60% of the original volume. Disruption of the LGF was accomplished by high-speed blenderization for 60 sec at 4°. Free and total acid phosphatase activity was measured respectively in the whole homog-

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enate, 10,000*g* supernate, and large-granule fraction without and with the incorporation of 0.01% Triton X-100 into the substrate, sodium β-glycerophosphate (11). Protein contents were determined by the Lowry method (12), and acid β-glycerophosphatase activity was expressed as the mg of inorganic phosphate liberated/100 mg of protein/10 min.

Deiodination of ^{131}I-triglyceride. Separation of free ^{131}I and lipid-bound ^{131}I components of the total hepatic radioactivity was performed as previously described (6) by a modification of the method of Turner (13). Briefly, following lipid emulsion administration *in vivo* the total liver was rapidly excised, washed in cold 10% KI solution. An aliquot of the mixture was diluted with carrier iodide solution, and then 40% trichloroacetic acid (TCA) was added to precipitate the lipid-protein complex. After centrifugation, the supernate containing free I^{131} was discarded, and the lipid-bound ^{131}I labeled precipitate was subjected to an additional KI wash, centrifugation, and decantation in order to remove free ^{131}I. Hepatic deiodination *in vivo* was expressed as the percent of localized radioactivity present in the free or deiodinated form (% deiodinated) and the μg of ^{131}I-triglyceride by the different hepatic subcellular fractions was evaluated by incubating an aliquot of either whole homogenate, 10,000*g* supernate, or large-granule fraction with 480 μg of the ^{131}I-triglyceride emulsion. Samples were oscillated (90 cpm) at 37° in a Dubnoff metabolic apparatus. At the end of the incubation period, separation of free ^{131}I and lipid-bound ^{131}I was performed similar to above except that the 10% KI solution added prior to TCA contained 1% albumin to ensure complete lipid-protein precipitation. *In vitro* deiodination was expressed as the percent deiodinated per hour and the μg of ^{131}I-triglyceride deiodinated/100 mg of protein/hr.

Data analysis. Determination of blood and tissue ^{131}I was performed with a deep-well crystal scintillation counter (Nuclear-Chicago, Des Plaines, IL). All data were compared for statistical significance at the 95% confidence level. Tabulated data are expressed as the mean ± standard error.

TABLE I. HEPATIC LOCALIZATION AND DEIODINATION IN VIVO OF ^{131}I-LIPID EMULSION.[a]

Post-injection Time (min)	Localization[b]		Deiodination[c]	
	mg/g	%ID/TO	% Deiod.	μg Deiod./g
15	2.56±0.06	69.9±2.4	4.2±0.9	100±24
30	3.08±0.25	81.6±3.2	6.9±0.8	212±32
60	2.04±0.11	49.8±3.3	13.2±0.6	310±25

[a] Data derived from four rats/point are presented as the mean ± standard error of each time following administration of "RE-test-lipid emulsion" at a dose of 30 mg/100 g of body wt.

[b] Localization is expressed as the mg of ^{131}I-triglyceride/g of liver and the percent of the injected dose (% ID) per total organ (TO).

[c] Deiodination is expressed as the percent of ^{131}I-triglyceride deiodinated and the μg deiodinated/g of liver.

Results. Hepatic localization and deiodination in vivo. The half-time ($t_{1/2}$) for phagocytic clearance of the lipid particles from the circulation at the 30 mg/100 g dose was 7.9 (±0.6) min as determined in six rats. The total hepatic localization of 69.9% of the injected dose at 15 min (Table I) correlates well with the clearance rate and reflects the selective uptake by the liver. Uptake by the spleen and lungs, two other major RE cell-containing organs, at this time was 3.1 and 0.4%, respectively. Maximum hepatic localization at 30 min (81.6%) was followed by rapid loss of radioactivity from the liver, presumably as free ^{131}I, so that by 60 min only 49.8% remained. The percent of hepatic radioactivity present in the free ^{131}I or deiodinated form increased progressively with time. The 212 μg of ^{131}I-triglyceride deiodinated/g of liver at 30 min represented a degradative rate of 232 μg of ^{131}I-triglyceride deiodinated/100 mg of hepatic protein/hr.

In vitro deiodination by hepatic fractions. In order to determine the locus of intracellular lipid degradation within the liver, ^{131}I-triglyceride particles were incubated *in vitro* with different hepatic subcellular fractions (Table II.) The highest deiodination was observed in the whole homogenate, supernate, and large-granule fraction, respectively. The highest specific activity of

TABLE II. COMPARATIVE IN VITRO DEIODINATION OF ^{131}I-LIPID EMULSION BY HEPATIC SUBCELLULAR FRACTIONS.[a]

Hepatic fraction[b]	% Deiodinated/hr[c]	μg Deiodinated/100 mg protein/hr[c]
Whole homogenate	0.655 ± 0.042	17.2 ± 1.1
Supernate	0.108 ± 0.028	5.6 ± 1.4
Large-granule	0.435 ± 0.023	67.2 ± 3.5

[a] Data are presented as the mean ± standard error of 20 determinations for each fraction prepared from the livers of four rats. A 480 μg dose of ^{131}I-triglyceride particles was incubated with the three hepatic fractions for 120 min with shaking (90 cpm) at 37°.

[b] Hepatic fractions were prepared from a 10% homogenate of rat liver in 0.25 *M* sucrose. The supernate and large-granule fractions are the 10,000 *g* supernate and sediment, respectively.

[c] Deiodination expressed as the percent of the dose deiodinated per hr and the μg of ^{131}I-triglyceride deiodinated/100 mg of protein/hr for the three fractions were significantly ($P < 0.01$) different by analysis of variance.

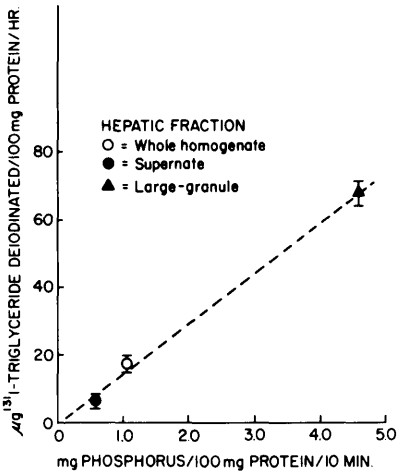

FIG. 1. Relationship of *in vitro* triglyceride deiodination to total acid phosphatase activity in hepatic subcellular fractions. Hepatic fractions were prepared from a 10% homogenate of rat liver in 0.25 *M* sucrose. Deiodination expressed as the μg of ^{131}I-triglyceride deiodinated/100 mg of protein/hr is presented as the mean ± standard error of 20 determinations for each fraction prepared from the livers of four rats. Total acid phosphatase activity is expressed as the mg of phosphorous formed/100 mg of protein/10 min.

deiodination was present in the LGF (67.2 μg of ^{131}I-triglyceride/100 mg of protein/hr). Analysis of total acid phosphatase, a lysosomal marker enzyme, and deiodination activity of the three hepatic fractions (Fig. 1) demonstrated a highly significant ($P < 0.01$) correlation between the two parameters. The highest specific activities of both acid phosphatase and lipid degradation were localized within the hepatic large-granule fraction. Evaluation of the time course of deiodination by the LGF (Table III) revealed a marked lag of activity for the intact fraction such that the rate at 7.5 and 15 min was significantly ($P < 0.05$) less than at 60, 120, and 180 min. This lag was eliminated by prior disruption of the LGF by high-speed blenderization. Lysosome disruption was verified by the free to total acid phosphatase ratios which were 0.10 and 0.85 for the intact and disrupted fractions, respectively, prior to incubation and 0.96 after 120 min of incubation for both fractions.

The addition of phosphate buffer to the large-granule fraction in 0.25 *M* sucrose permitted manipulation of the hydrogen ion concentration. In this respect, deiodination in the LGF increased significantly ($P < 0.05$) from 69.0 (±3.7) to 155.0 (±11.2) μg of ^{131}I-triglyceride deiodinated/100 mg of protein/hr when the pH was lowered from 7.0 to 5.0. Adjusting the pH did not significantly increase the deiodination of lipid in phosphate-buffered 0.25 *M* sucrose alone without the LGF, i.e., 0.37 (±0.16) at pH 7.0 as compared to 0.53 (±0.10) at pH 5.0, when expressed as the μg of ^{131}I-triglyceride deiodinated/hr. In these experiments, each group consisted of 12 determinations.

Discussion. Previous studies in animals and man have adequately demonstrated that the "RE-test-lipid emulsion" utilized in the present investigation is localized exclusively within macrophages, particularly hepatic Kupffer cells, following intravenous administration (6, 7, 14). Following ingestion, the phagocytized ^{131}I-triglyceride particles are degraded within the liver, and the free ^{131}I is subsequently released into the blood (6). While the vascular clearance of a comparable lipid dose (30 mg/100 g of body wt) is slower in rats ($t_{1/2}$ = 7.9 min) than in dogs ($t_{1/2}$ = 2.8 min) (6), the pattern of intense

TABLE III. EFFECT OF LGF DISRUPTION ON THE TEMPORAL PATTERN OF IN VITRO DEIODINATION OF ^{131}I-TRIGLYCERIDE.[a]

Incubation time (min)	Deiodination (μg/100 mg protein/hr)[b]	
	Intact LGF	Disrupted LGF
7.5	28.3 ± 5.0[c]	62.6 ± 9.8
15	34.6 ± 6.5[c]	74.6 ± 11.7
30	58.6 ± 9.6	67.8 ± 10.9
60	68.3 ± 7.4	70.5 ± 7.7
120	67.2 ± 3.5	73.0 ± 5.9
180	59.2 ± 5.4	62.0 ± 4.8

[a] Data are presented as the mean ± standard error of 10–20 determinations at each time. Disruption of the hepatic large-granule fraction was accomplished by high-speed blenderization for 60 sec at 4°.

[b] Deiodination is expressed as the μg of ^{131}I-triglyceride deiodinated/100 mg of LGF protein/hr.

[c] Values were significantly ($P < 0.05$) different by Student's unpaired t test from those both at other time intervals for the intact LGF and at the same time intervals for the disrupted LGF.

hepatic localization, progressive ^{131}I-triglyceride deiodination, and rapid release of free ^{131}I is common to both species.

Since rat livers are capable of ^{131}I-triglyceride degradation *in vivo*, hepatic subcellular fractions were prepared in order to evaluate the cellular component responsible for this degradation. Although the fractions were prepared from whole liver rather than the Kupffer cell population alone, the highest specific activity of deiodination *in vitro* was localized in the hepatic large-granule fraction, which is composed primarily of lysosomes and mitochondria (10). The microsome containing 10,000*g* supernate fraction was almost devoid of activity. There was a highly significant correlation between the total acid phosphatase and deiodinative activity of the different hepatic fractions. Acid phosphatase is an established marker enzyme for lysosomes, which are not only plentiful within macrophages but also vital for the degradative function of reticuloendothelial cells (8, 9). Further implications of lysosomes as the organelles responsible for lipid degradation is the finding that *in vitro* deiodination by the hepatic LGF demonstrated both a latency of activation which was overcome by lysosome disruption and an acid pH optimum characteristic of lysosomal lytic enzymes. In addition, previous studies have reported the presence of phagocytized lipid within lysosomes (15) as well as the existence of lysosomal lipases with an acid pH optimum (16, 17). The low degradative activity observed *in vitro* as compared to the *in vivo* activity appears to be due to the dilution of lysosomal enzymes within the relatively large incubation volume.

Previous studies utilizing ^{131}I-labeled aggregated albumin have concluded that lysosomes are responsible for the intracellular degradation of this material following phagocytic uptake (18, 19). However, similar to the present findings, the identity of the actual lytic enzyme responsible for the protein deiodination was not known. While it is possible that peroxisomes, which are present in the hepatic large-granule fraction, are involved in ^{131}I-triglyceride deiodination, it has been shown that peroxisomes contain oxidative enzymes such as catalase with an alkaline pH optimum (20) rather than the acid pH optimum (8).

Similar to the lipid degradative activity of the hepatic LGF demonstrated on the present study, Filkins (21) has recently reported that this subcellular fraction contains the endotoxin detoxifying activity of liver. Endotoxin is known to be phagocytically removed from the circulation primarily by hepatic macrophages (22). In addition, the endotoxin detoxifying activity of liver displays both a latency of activation, i.e., probably requires disruption of lysosomes to release lytic enzymes *in vitro*, (21) and an acid pH optimum (23). Both of these properties are consistent with the ^{131}I-triglyceride degradative activity of the hepatic LGF observed in the present study. The similarity of these two systems coupled with the need to be able to critically assess the degradative aspects of the RES suggests that the present technique utilizing the radiolabeled lipid particles may provide an accurate evaluation of the two critical RES functions, i.e., phagocytosis (particle clearance) and degradation (deiodination). Additionally, the findings suggest that similar to endotoxin, lipid degradation within macrophages seems to be mediated by lysosomes.

Summary. Radiolabeled particulate lipid administered intravenously was cleared from the circulation and localized primarily within the liver. The phagocytized ^{131}I-triglyceride was degraded with the subsequent release of free ^{131}I from the liver. Incubation of the ^{131}I-triglyceride emulsion *in vitro* with different hepatic subcellular fractions revealed that the lysosome rich large-granule fraction contained the highest specific deiodinative activity. This observation coupled with the findings on latency of activation and an acid pH optimum, support the concept that the lysosomes are critically involved in the degradation of the particulate phagocytized lipid in the liver.

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