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Synthesis of hepatic lipase in liver and extrahepatic tissues[1]

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Abstract Immunoprecipitations of hepatic lipase from pulse-labeled rat liver have demonstrated that hepatic lipase is synthesized in two distinct molecular weight forms, HL-I (M_r = 51,000) and HL-II (M_r = 53,000). Both forms are immunologically related to purified hepatic lipase, but not to lipoprotein lipase. HL-I and HL-II are also kinetically related and represent different stages of intracellular processing. Glycosidase experiments suggest that HL-I is the high mannose microsomal form of the mature, sialyated HL-II enzyme. Hepatic lipase activity was detected in liver and adrenal gland but was absent in brain, heart, kidney, testes, small intestine, lung, and spleen. The adrenal and liver lipase activities were inhibited in a similar dose-dependent manner by hepatic lipase antiserum. Immunoblot analysis of partially purified adrenal lipase showed an immunoreactive band co-migrating with HL-II at 53,000 daltons which was absent in a control blot treated with preimmune serum. Adrenal lipase and authentic hepatic lipase yielded similar peptide maps, confirming the presence of the lipase in adrenal gland. However, incorporation of L-[^{35}S]methionine into immunoprecipitable hepatic lipase was not detected in this tissue. In addition, Northern blot analysis showed the presence of hepatic lipase mRNA in liver but not adrenal gland. The presence of hepatic lipase in adrenal gland in the absence of detectable synthesis or messenger suggests that hepatic lipase originates in liver and is transported to this extrahepatic site. —**Doolittle, M. H., H. Wong, R. C. Davis, and M. C. Schotz.** Synthesis of hepatic lipase in liver and extrahepatic tissues. *J. Lipid Res.* 1987. **28:** 1326–1334.

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Two major enzymes involved in triacylglycerol and phospholipid metabolism of circulating lipoproteins are hepatic lipase and lipoprotein lipase. Classically, hepatic lipase activity has been distinguished from lipoprotein lipase activity on the basis of its differing protein cofactor and salt requirement. Although both hepatic and lipoprotein lipase catalyze the hydrolysis of mono-, di-, and triacylglycerol substrates as well as a number of phospholipids in vitro, their natural lipoprotein substrate specificities are distinctive. For example, lipoprotein lipase specifically hydrolyzes triacylglycerols from apoC-II-containing chylomicrons and VLDL. In contrast, hepatic lipase appears to prefer lipoproteins deficient in apoC-II such as chylomicron remnants, IDL, and HDL_2 (for reviews, see refs. 1, 2).

The in vivo functions of hepatic lipase are unclear. Studies in which hepatic lipase activity in vivo is blocked by the injection of specific antibodies have suggested that hepatic lipase is the critical enzyme for conversion of IDL to LDL (3–5), and that it may process apoB-48-containing chylomicron remnants for uptake by the liver (6) as well as convert HDL_2 to HDL_3 (2). This latter function may be important in mediating reverse cholesterol transport, a process thought to protect extrahepatic tissues from cholesterol accumulation (7).

Hepatic lipase has been purified from human (8), canine (9), and rat liver (10, 11). The enzyme which we have purified from heparin perfusates of rat liver migrates on SDS polyacrylamide gels as one major band with an apparent molecular weight of 53,000 (12). Recently, we have isolated and sequenced the full-length rat liver hepatic lipase cDNA clone (13). Hepatic lipase appears to be a classical secretory protein containing a 22-amino acid hydrophobic leader sequence and two potential N-linked glycosylation sites. Based on the derived amino acid sequence, the molecular weight of the mature unglycosylated protein is 53,222.

Direct evidence for the synthesis of hepatic lipase in any tissue has not been reported. Hepatic lipase activity is

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; HEPES, N-2-hydroxyethylpiperazine-N-ethane-sulfonic acid; PMSF, phenylmethylsulfonyl fluoride; KRB, Krebs-Ringer bicarbonate; PBS, phosphate-buffered saline; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Staph A, crude insoluble Protein A from lyophilized *Staphylococcus aureus* cells (Cowan strain).

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secreted by primary cultures of rat and chicken liver parenchymal cells (14-16) and a human hepatoma cell line (17). This lipase activity binds to liver nonparenchymal cells (18, 19) and has been localized by immunocytochemistry to liver endothelium (20), presumably the functional location of the mature enzyme. Hepatic lipase activity, inhibitable by hepatic lipase-specific antibodies, has also been detected in adrenal gland (21-24) and ovary (23, 25, 26). The present study utilizes immunoprecipitation, immunoblotting, and mRNA hybridization techniques to: *1*) elucidate the steps in liver intracellular processing of newly synthesized hepatic lipase; *2*) establish the presence of hepatic lipase in adrenal gland; and *3*) determine the capability of adrenal gland to synthesize this enzyme.

EXPERIMENTAL PROCEDURES

Materials

Peptide:N-glycosidase-F was purchased from Genzyme Corporation, endoglycosidase-H from Boehringer-Mannheim Biochemicals, goat anti-rabbit IgG horseradish peroxidase conjugate from Bethesda Research Laboratories, ENHANCE® from New England Nuclear, L-[^{35}S]methionine, Na[^{125}I]iodide, and glycerol tri[9,10(n)-^{3}H]oleate from Amersham and ICN Radiochemicals. HEPES, PMSF, Triton X-100, N-lauroyl sarcosine, crude insoluble Protein A (Staph A), and *Staphylococcus aureus* V8 protease were obtained from Sigma.

Animals

Male Sprague-Dawley rats, 250-350 g, were maintained on a 10-hr dark/14-hr light cycle. Unrestricted access to standard Purina Laboratory Chow and water was provided at all times.

Tissue labeling

Livers were pulse-labeled with [^{35}S]methionine utilizing a recirculating perfusion system. After laparotomy, the hepatic portal vein was cannulated and perfused at 2 ml/min per g liver with KRB-supplemented with 10 mM HEPES (KRB-HEPES[2]). After 6 min, a recirculating perfusion at 5 ml/min per g liver was initiated utilizing a 25-ml reservoir of KRB-HEPES. [^{35}S]Methionine (1 mCi/g liver) was introduced into the reservoir with continual stirring. Following a 15-min pulse, the liver was homogenized in 10 ml/g liver of ice-cold 0.25 M sucrose, 1 mM PMSF, 0.1 M Tris-HCl, pH 7.4. An enriched microsomal fraction was obtained by subjecting the postmitochondrial supernatant fraction to 143,000 g_{max} for 30 min. Typically, 30-50% of the [^{35}S]methionine label was incorporated into acid-precipitable material; approximately 50% of the incorporated label was recovered in the microsomal fraction. Just prior to immunoprecipitation, the microsomal fraction was treated with detergent, yielding a final concentration of 20 mg/ml microsomal protein, 3% Triton X-100, 0.1% N-lauroyl sarcosine, 1 mM PMSF in PBS, pH 7.2.

Pulse-labeling experiments were carried out in vivo by injecting 5 mCi of [^{35}S]methionine into the iliolumbar vein of Nembutal-anesthetized animals. Liver lobes were progressively tied off and removed after 5, 10, 30, and 90 min. Each lobe was immediately homogenized in 10 ml/g liver lysis buffer (3% Triton X-100, 0.1% N-lauroyl sarcosine, 1 mM PMSF in PBS). Intact nuclei and debris were removed by centrifugation (48,200 g_{max}, 30 min). Ninety minutes after the [^{35}S]methionine injection, small intestine, kidney, adrenal glands, and testes were also removed and homogenized as above.

Adrenal glands were also pulse-labeled in vitro. Forty adrenal glands (total weight, 1 g) were removed, decapsulated, and sliced. The slices were incubated in 10 ml of KRB-HEPES for 5 min before adding 3 mCi of [^{35}S]methionine. Following a 20-min incubation in isotope, the adrenal gland slices were washed once in ice-cold normal saline and homogenized in 5 ml of lysis buffer. The specific activity of label incorporated into acid-precipitable material was 2.7×10^6 dpm/mg protein, 10-fold greater than liver pulse-labeled 90 min in vivo.

Immunoprecipitation

Tissue lysates were pretreated with Staph A (10 μl of 10% slurry per mg of lysate protein) for 15 min at 4°C. Staph A was pelleted (12,000 g_{max}, 5 min) and the appropriate titer of hepatic lipase immune serum was added to the supernatant fraction (see Other Methods). Antibody-antigen complexes were allowed to form for 1 hr at 4°C with continual mixing. A quantity of Staph A to sufficiently bind all IgG was added, incubated for 15 min at 4°C, and pelleted. The pellet was washed three times with PBS containing 0.1% N-lauroyl sarcosine. The Staph A-antibody-antigen complexes were dissociated by adding 2% SDS, 0.1 M Tris-HCl, pH 6.8, and boiled for 2 min. The samples were centrifuged to remove the insoluble Staph A, and glycerol and bromphenol blue were added to the supernatant fractions for SDS PAGE (see Other Methods).

Identity of immunoprecipitable bands was established by pretreating hepatic lipase antiserum with pure, unlabeled hepatic or lipoprotein lipase. One μg of hepatic or lipoprotein lipase was added to each μl of antiserum and incubated for 60 min at 4°C in the presence of 1 mM PMSF. The pretreated antiserum was then utilized for immunoprecipitation.

[2]In all procedures using KRB-HEPES, the medium was maintained at 37°C and gassed continuously with O_2-CO_2 95:5.

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Glycosidase experiments

^{35}S-labeled HL-I and HL-II were immunoprecipitated from liver pulse-labeled 30 min in vivo as described above. Peptide:N-glycosidase-F and endoglycosidase-H were added to separate immunoprecipitates containing 0.1% SDS, 0.7% Triton X-100 in 0.5 ml of PBS to a final concentration of 6 and 5 mU/ml, respectively. Control samples received no glycosidases but were otherwise handled identically. All samples were incubated for 24 or 48 hr at room temperature. Control and glycosidase-treated samples were immunoprecipitated a second time and prepared for SDS PAGE as described above.

Peptide mapping

A one-dimensional peptide fragment map was generated by the method of Cleveland et al. (27). Adrenal lipase was partially purified by heparin-Sepharose chromatography under the same conditions used to bind liver hepatic lipase (12). Adrenal lipase and purified hepatic lipase were radiolabeled with Na[^{125}I]iodide (28), isolated as single bands from SDS 7-14% gradient polyacrylamide gels, and subjected to a second 12-18% SDS gel containing 30 μg of V8 protease in the sample wells. Partial proteolysis was obtained by stopping electrophoresis of the sample zone at the stacking/separating gel interface for 60 min. The resulting peptide fragments were separated by resuming electrophoresis of the sample zone through the separating gel. The gels were dried and subjected to autoradiography using standard procedures.

Immunoblot and Northern analysis

Immunoblots were performed by the method of Towbin, Staehelin, and Gordon (29) except for the use of Schleicher and Schuell nitrocellulose sheets (pore size, 0.2 μm) and CBS Scientific electrophoretic blotting unit. Transfer was conducted for 18 hr at 30 v after which the nitrocellulose sheets were incubated in 10 mM Tris, pH 7.4, containing 0.15 M NaCl, 3% bovine serum albumin (buffer A). Following a 1-hr incubation at 37°C, hepatic lipase antiserum in buffer A (1:2000 dilution) was added and incubated for an additional 1 hr at room temperature. Identical blots were similarly probed with preimmune serum. The blots were washed three times with 10 mM Tris, pH 7.4, containing 0.15 M NaCl and 0.05% Triton X-100 (buffer B). Horseradish peroxidase-conjugated goat antirabbit IgG in buffer A (1:2000 dilution) was added to the blots for 2 hr. The blots were washed three times with buffer B and processed for color reaction following the manufacturer's (BRL) instructions.

Total RNA was prepared from rat tissues by homogenization in guanidinium thiocyanate followed by centrifugation over a cesium chloride cushion (30); poly (A+) RNA was selected by oligo(dt)-cellulose chromatography. RNA formaldehyde-agarose gel electrophoresis and blotting with a ^{32}P-labeled hepatic lipase cDNA probe were carried out as described elsewhere (13).

Other methods

Rat hepatic lipase was purified from liver heparin perfusate as described elsewhere (12). Antiserum to rat hepatic lipase was prepared in male New Zealand White rabbits by standard procedures (31) utilizing 40 μg of purified hepatic lipase for initial and subsequent subcutaneous injections. The titer of the antiserum was determined by measuring the volume of antiserum required to immunoprecipitate hepatic lipase quantitatively from a known amount of liver or microsomal lysate. The immunoprecipitation was considered quantitative when additional hepatic lipase was not brought down from the lysate by a second incubation in antibody. The titers of immune serum were: liver microsomal lysate, 8 μl of antiserum/mg of protein; liver and other tissue lysates, 1 μl of antiserum/mg of protein.

Tissue samples used for hepatic lipase activity were prepared as 20% (w/v) homogenates in 0.15 M NaCl, 0.005 M barbital buffer, pH 7.4, containing 5 U/ml heparin. All homogenates were cleared by centrifugation at 48,200 g_{max} for 30 min at 4°C. Lipase assays utilizing glycerol tri[^{3}H]oleate (sp act 4.3 × 10^3 cpm/nmol) were performed as previously described (11).

SDS PAGE was performed as described previously (12). Samples prepared for immunoblot analysis contained 1% β-mercaptoethanol, while β-mercaptoethanol was omitted from immunoprecipitated samples. Gels for fluorography were fixed in 40% methanol, 5% tricholoacetic acid for 1 hr and incubated in ENHANCE® following manufacturer's (NEN) instructions prior to drying and autoradiography at −80°C.

Protein values for tissue homogenates were determined by the method of Lowry et al. (32) and values for purified hepatic lipase by the Coomassie blue dye binding assay (33). Coomassie blue protein assays correlated more positively to mass as determined by amino acid analysis than other methods of protein determination.

RESULTS

Liver synthesis of hepatic lipase

The first set of experiments was designed to determine the identity of bands immunoprecipitated by antiserum raised against purified hepatic lipase. A crude microsomal fraction was isolated from rat liver perfused 15 min with [^{35}S]methionine. Immunoprecipitates from the labeled microsomes showed the presence of two molecular weight species, HL-I (51,000 daltons) and HL-II (53,000 daltons), not present using preimmune serum (**Fig. 1A**). Addition of unlabeled hepatic lipase during immunoprecipitation

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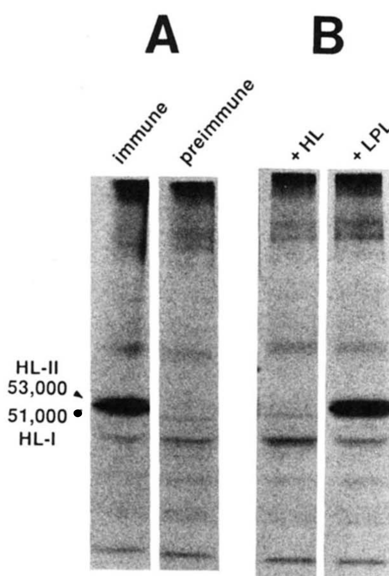

Fig. 1. Synthesis of rat liver hepatic lipase. Microsomes were isolated from rat livers pulse-labeled 15 min with [^{35}S]methionine. Immunoprecipitates were subjected to 7-14% gradient SDS PAGE and exposed to Cronex® film for 5 days: Panel A, a fluorogram of immunoprecipitated bands using immune or preimmune serum; Panel B, immunoprecipitated bands utilizing immune serum preincubated with unlabeled hepatic (+HL) or lipoprotein lipase (+LPL) (see Experimental Procedures). The position of migration of the two proteins that are specifically precipitated by immune serum and competed by unlabeled hepatic lipase are indicated (HL-I and HL-II, 51,000 and 53,000 daltons, respectively). H-II also marks the migration position of purified hepatic lipase.

completely eliminated the precipitation of both molecular weight species whereas addition of unlabeled lipoprotein lipase had no effect (Fig. 1B). In addition, antibody to hepatic lipase did not cross-react with purified lipoprotein lipase on immunoblots (data not shown). Together, these data indicate that both HL-I and HL-II are forms of hepatic lipase.

A pulse-labeling experiment was carried out in vivo to examine the possibility that HL-I and HL-II were kinetically related (**Fig. 2**). [^{35}S]Methionine was injected intravenously, and liver lobes were tied off progressively after 5, 10, 30, and 90 min. Immunoprecipitates of hepatic lipase revealed that at early time points (5 and 10 min), only HL-I, M_r = 51,000 was labeled; after 30 min, both forms were labeled in roughly equal proportions; and after 90 min, only HL-II, M_r = 53,000 was labeled. Similar results were obtained using microsomes prepared from perfused livers following a 15-min pulse and 30-min chase (data not shown). HL-II co-migrated on SDS polyacrylamide gels with heparin-releasable hepatic lipase and probably represents the mature form of the enzyme. The data suggest that HL-I represents an intermediate in the processing of hepatic lipase to the mature enzyme.

Since hepatic lipase contains potential N-linked glycosylation sites (13), HL-I is most likely the N-linked high mannose microsomal form which is processed to the mature, sialyated HL-II enzyme. To test this possibility, ^{35}S-labeled HL-I and HL-II were incubated with endoglycosidase-H, an enzyme that preferentially cleaves the high mannose groups from N-linked glycoproteins (34, 35). After a 24-hr incubation with endoglycosidase-H, HL-I was cleaved into two lower molecular weight forms, designated HL-I_a and HL-I_b (**Fig. 3,** lane B). Longer incubation with endoglycosidase-H cleaved HL-I_a further to HL-I_b (Fig. 3, lane C). This finding suggests that the two potential N-glycosylation sites in hepatic lipase are both utilized, HL-I_a representing cleavage of one of the two N-linked high mannose sugar groups, and HL-I_b representing complete cleavage of both sugar moieties. In addition, incubation with peptide:N-glycosidase-F, an enzyme that hydrolyses both high mannose and sialyated sugar moieties of N-linked glycoproteins (36, 37), reduced HL-II and HL-I to one band that co-migrated with HL-I_b (Fig. 3, lane D). Combined, these findings suggest that HL-I and HL-II differ only in carbohydrate composition and constitute different degrees of intracellular processing.

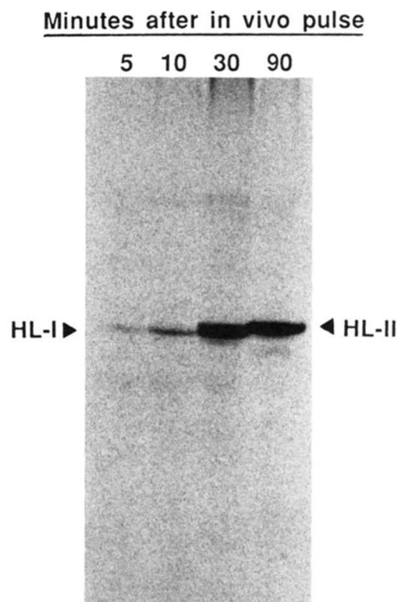

Fig. 2. Determination of precursor-product relationship between HL-I and HL-II. At the indicated times, liver lobes were tied off and homogenates were prepared as described in Experimental Procedures and Results. Homogenates were treated with preimmune serum at a titer equivalent to antiserum (see Experimental Procedures). After incubating for 1 hr at 4°C, Staph A was added to precipitate preimmune-antigen complexes. The preimmune-treated homogenates were then utilized for immunoprecipitations with hepatic lipase antiserum as described in Experimental Procedures and the legend to *Fig. 1.* The position of migration of HL-II (M_r = 53,000) and HL-I (M_r = 51,000) are indicated.

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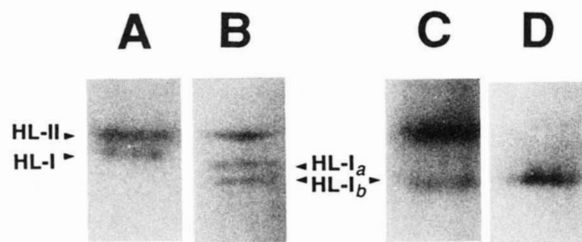

Fig. 3. Differences in glycosylation of ^{35}S-labeled HL-I and HL-II determined by endoglycosidase-H and peptide: N-glycosidase-F cleavage. Homogenates prepared from liver pulse-labeled 30 min in vivo were treated with preimmune serum and immunoprecipitated with hepatic lipase antiserum (see legend to Fig. 2). These immunoprecipitates were then incubated with glycosidases for 24 or 48 hr. All samples were immunoprecipitated a second time and subjected to SDS PAGE and fluorography using XAR-5 film for 14–33 days to optimize exposures (see Experimental Procedures): lane A, buffer only; lane B, endoglycosidase-H for 24 hr; lane C, endoglycosidase-H for 48 hr; lane D, peptide:N-glycosidase-F for 48 hr. The migration position of HL-I, HL-II, and the two cleavage products of HL-I ($HL\text{-}I_a$ and $HL\text{-}I_b$) are indicated.

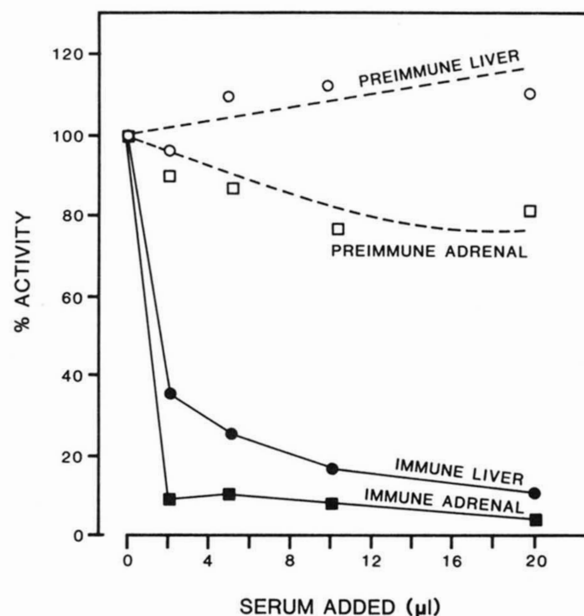

Fig. 4. Inhibition of hepatic and adrenal lipase activity by antiserum to hepatic lipase. Lipase was partially purified by heparin-Sepharose chromatography of homogenates prepared from 0.5 g of liver tissue and 0.4 g of adrenal gland tissue (see Experimental Procedures). Active fractions eluted with 0.9 M NaCl were pooled and samples were incubated with various amounts of immune serum (closed symbols) or preimmune serum (open symbols) in a total volume of 200 µl. Following a 1-hr incubation at 4°C, samples were assayed for lipase activity as described in Experimental Procedures. The 100% value for hepatic and adrenal gland lipase activity was 22.6 and 13.9 mU/ml, respectively.

Hepatic lipase in adrenal gland

To determine whether extrahepatic tissues contain hepatic lipase activity, homogenates prepared from brain, heart, adrenal gland, kidney, liver, testes, small intestine, lung, and spleen were assayed for lipase activity in the presence of high salt. Only liver and adrenal gland displayed activities that were dependent on homogenate concentration. Adrenal gland contained roughly one-quarter the specific activity (mU/mg cellular protein) of liver (**Table 1**). In addition, hepatic lipase activity in adrenal gland and liver was inhibited by hepatic lipase antiserum, but not by preimmune serum, in a similar dose-dependent manner (**Fig. 4**). Addition of 3% Triton X-100 to homogenates during their preparation inhibited lipase activity from liver and adrenal gland but uncovered a lipase activity in small intestine, approximately sixfold greater than liver on a protein mass basis. However, whereas liver and adrenal lipase activities bound and eluted from heparin-Sepharose in a similar quantitative manner, intestinal lipase activity bound only poorly and was not recovered after 1 M NaCl elution. On the bases of activity in high salt, inhibition by immune serum, and characteristic binding to heparin-Sepharose, it was concluded that of the tissues examined, only adrenal gland and liver contained hepatic lipase activity.

TABLE 1. Tissue distribution of hepatic lipase activity

Tissue	Specific Activity
	mU/mg
Liver	8.5 ± 1.7
Adrenal gland	2.2 ± 0.1
Other tissues	ND[a]

Tissues were removed and homogenized in 5 mM barbital buffer, pH 7.2, containing 1 mM PMSF and 5 U/ml heparin. Tissue protein over a 10-fold range was assayed for hepatic lipase activity as described in Experimental Procedures. Each value (± SD) is an average of four separate determinations performed in duplicate. One milliunit is defined as 1 nmol of free fatty acid hydrolyzed per min.

[a]Brain, heart, small intestine, kidney, lung, spleen, and testes contained no detectable hepatic lipase activity.

Immunoblot analysis was utilized to investigate the identity of the adrenal gland lipase. Adrenal lipase, partially purified from homogenates by heparin-Sepharose chromatography, and purified hepatic lipase were subjected to SDS PAGE, transferred to nitrocellulose paper, and probed with immune and preimmune serum (**Fig. 5**). Partially purified adrenal lipase showed an immunoreactive band (lane B) not present in the preimmune blot (lane C), which co-migrated with purified hepatic lipase at a M_r = 53,000 (lane A). Nonspecific bands were eliminated (lane D) following further purification of the adrenal lipase through the chromatography steps utilized to purify the liver enzyme (12). In contrast, utilizing equal units of intestinal lipase activity, immunoreactive bands corresponding to hepatic lipase were absent (data not shown).

To confirm the identity of adrenal lipase as hepatic lipase, a peptide map was generated by the method of

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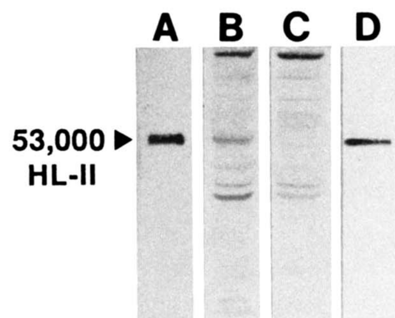

Fig. 5. Immunoblot analysis of hepatic and adrenal lipase: lane A, purified hepatic lipase (65 ng) probed with hepatic lipase antiserum; lane B, adrenal lipase (1 mU), partially purified by heparin-Sepharose chromatography, and probed with hepatic lipase antiserum; lane C, identical to lane B but probed with preimmune serum; lane D, adrenal lipase, further purified by chromatography through heparin-Sepharose, hydroxylapatite, and dextran-sulfate-Sepharose (12), and probed with hepatic lipase antiserum. The migration position of HL-II (M_r = 53,000) is indicated.

Cleveland et al. (27). Partially purified adrenal lipase and purified hepatic lipase were radioiodinated, isolated as single bands from SDS polyacrylamide gels, and subjected to a second SDS gel containing V8 protease. The peptide fragments generated by V8 protease cleavage of liver and adrenal gland lipase co-migrated (**Fig. 6**). These data along with the immunoblot analysis are strong evidence that adrenal gland contains authentic hepatic lipase.

The capability of adrenal gland and other extrahepatic tissues to synthesize hepatic lipase was examined. Although liver synthesis of hepatic lipase was clearly seen, incorporation of [^{35}S]methionine into immunoprecipitable hepatic lipase was not detected in small intestine, kidney, or testes (**Fig. 7A**). In addition, immunoprecipitation of hepatic lipase from adrenal gland slices pulse-labeled in vitro failed to detect synthesized hepatic lipase (Fig. 7B). The inability of adrenal gland to synthesize hepatic lipase was further corroborated by probing RNA blots with ^{32}P-labeled rat liver hepatic lipase cDNA. Although a 1,750-bp band hybridizing with the hepatic lipase cDNA probe was observed in liver poly(A+)RNA, a comparable band in adrenal gland or small intestine was not seen even after utilizing 10 times the amount of RNA and almost 4 times the film exposure time (**Fig. 8A**). Similarly, in Northern blots using total RNA isolated from liver, adrenal gland, and kidney, a band hybridizing with the cDNA probe at 1,750 bp was detected only in liver (Fig. 8B).

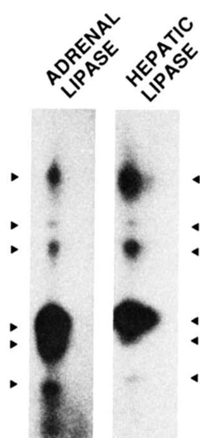

Fig. 6. One-dimensional peptide maps of hepatic and adrenal lipase. Peptide fragments were generated by V8 protease cleavage as described in Experimental Procedures and Results. Arrowheads indicate positions of ^{125}I-radiolabeled fragments after electrophoresis and autoradiography for 24-48 hr using XAR-5 film.

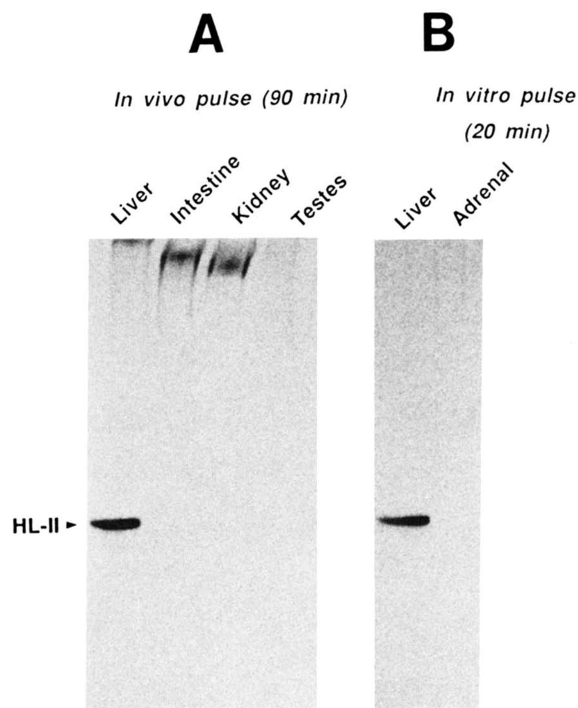

Fig. 7. Tissue synthesis of hepatic lipase. Panel A: Liver, small intestine, kidneys, and testes were excised from a rat 90 min after it received an intravenous dose of 5 mCi of [^{35}S]methionine. The tissues were homogenized, treated with preimmune serum, and immunoprecipitated with hepatic lipase antiserum as described in legend to Fig. 2 and Experimental Procedures. Panel B: Immunoprecipitates of liver and adrenal gland homogenates using hepatic lipase antiserum. Forty adrenal glands were sliced and incubated with 3 mCi of [^{35}S]methionine for 20 min as described in Experimental Procedures. Liver was pulse-labeled in vivo for 90 min. As compared with liver, adrenal gland slices incorporated 10 times the amount of [^{35}S]methionine into an equal amount of acid-precipitable material.

DISCUSSION

Direct evidence that hepatic lipase is synthesized by rat liver is presented in this study. Immunoprecipitation of hepatic lipase in pulse and pulse/chase experiments as

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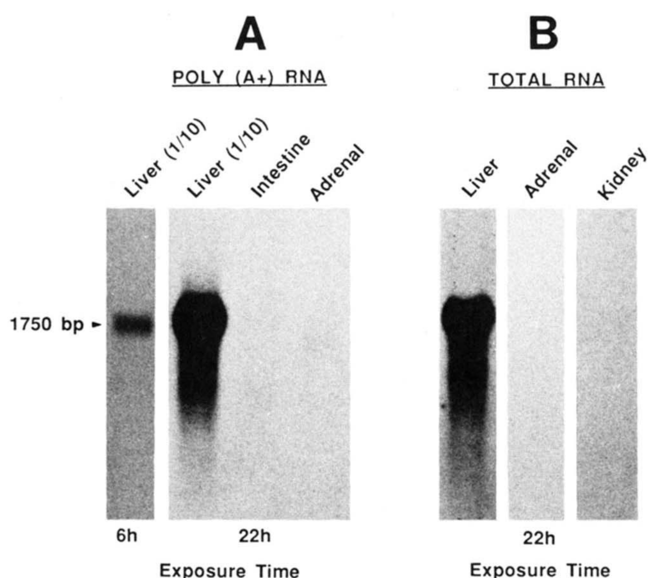

Fig. 8. Tissue distribution of hepatic lipase mRNA. Panel A: One μg of liver and 10 μg of small intestine, adrenal gland, and kidney poly(A+)RNA were subjected to agarose gel electrophoresis, transferred to nylon membranes, and probed with ^{32}P-labeled rat liver hepatic lipase cDNA as described in Experimental Procedures. Hybridization was assessed by autoradiography for 6 or 22 hr using XAR-5 film. Panel B: Ten μg of total RNA isolated from liver, adrenal gland and kidney was subjected to Northern blot analysis as described above. The migration position of hepatic lipase mRNA = 1,750 bp (13) is indicated.

well as treatment with glycosidases have established that synthesis of this extracellularly located enzyme follows that of a classical secretory glycoprotein, i.e., the early appearance of a lower molecular weight intermediate of glycosylation, most likely the high mannose form (HL-I), that is processed to the mature sialyated protein (HL-II). A protein identical in molecular weight to HL-II is released by heparin perfusion of livers, indicating that this form probably represents the liver endothelial-bound enzyme. This pathway proposed for hepatic lipase secretion is supported by the finding that tunicamycin and monesin inhibit the appearance of lipase activity in the medium of primary cultured rat hepatocytes (16), suggesting that newly synthesized hepatic lipase undergoes processing in the Golgi prior to secretion.

Of the extrahepatic tissues tested, only adrenal gland had a lipase activity similar to hepatic lipase. It has been suggested that tissues involved in steroidogenesis contain hepatic lipase, which would act to catalyze the influx of cholesteryl esters from exogenous lipoproteins, such as HDL_2 (38, 39). This view is upheld by the identification of hepatic lipase activity in bovine (24), rat and human adrenal cortex (22) and the corpus luteum of pseudopregnant female rats (25). However, we and others (23) were unable to measure any significant activity of the enzyme in testes, indicating that this proposed function is not mediated by hepatic lipase in all steroid-producing tissues.

An unexpectedly high lipase activity in rat small intestine homogenates prepared in Triton X-100 is consistent with recent evidence that monkey small intestine may contain hepatic lipase (Dr. W. Virgil Brown, personal communication). However, this rat intestinal lipase does not appear to be hepatic lipase, since: *1*) hepatic and adrenal lipase, but not intestinal lipase, is inhibited by Triton X-100 under our assay conditions; *2*) the intestinal lipase binds poorly to heparin-Sepharose and does not elute with 1 M salt; *3*) immunoblot analysis of intestinal lipase failed to show an immune positive band that comigrated with hepatic lipase. Perhaps the presence of hepatic lipase in monkey small intestine reflects species differences.

Adrenal lipase and hepatic lipase appear to be identical proteins, yielding similar molecular weights and one-dimensional peptide maps. The functional location of hepatic lipase in liver in believed to be on the sinusoidal face of liver endothelium (1, 2). At present, the functional location of hepatic lipase in adrenal gland is unknown. Perhaps the enzyme resides on the endothelial cells in adrenal cortex, since the sinusoidal histology of liver and adrenal cortex are similar and since one of the proposed functions of hepatic lipase (cholesteryl ester influx) is believed to occur in both organs.

The most striking finding in the present study was the inability of adrenal gland to synthesize hepatic lipase, even though this organ contains roughly one-quarter the activity of liver. Thus, we propose the novel hypothesis that hepatic lipase in adrenal gland originates in liver and is transported via the circulation to this extrahepatic site. A similar transport mechanism has been proposed for sterol carrier protein (SCP) (40), although unlike hepatic lipase, SCP does not contain a signal peptide and is incapable of translocation across microsomal membranes (41). Liver-derived hepatic lipase in circulation must presumably have the ability to target itself specifically to adrenal cortex. The mechanism by which this occurs is unknown. We postulate that secreted hepatic lipase is carried in plasma (possibly associated with HDL) and binds to specific receptors located on adrenal gland endothelium. Clarification of the precise steps in the transport of hepatic lipase will be addressed in future studies. ∎

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