Comparison of the triglyceride lipase of liver, adipose tissue, and postheparin plasma

John C. LaRosa,* Robert I. Levy, Herbert G. Windmueller, and Donald S. Fredrickson

Molecular Disease Branch, National Heart and Lung Institute, Bethesda, Maryland 20014

Abstract Heparin-released triglyceride lipase from three sources, adipose tissue, liver, and postheparin plasma, was compared. Heparin-released triglyceride lipase from liver differed in several major respects from that in adipose tissue. These differences included response to inhibitors and to high density lipoprotein in the incubation media. Heparin-released triglyceride lipase from liver, when compared with that from adipose tissue, was relatively inactive against lipoprotein substrates. The triglyceride lipase from postheparin plasma exhibited properties more like those of liver. These studies raise the possibility that triglyceride lipase in postheparin plasma may be heterogeneous and that levels of the enzyme in postheparin plasma may not accurately reflect the capacity for clearance of triglyceride from the plasma.

Supplementary key words lipoprotein lipase · postheparin lipolytic activity · high density lipoprotein · liver · adipose tissue

LIPOPROTEIN LIPASE, a triglyceride lipase present in adipose tissue, heart, lung, and other tissues, is thought to require lipoprotein-bound triglyceride as substrate. Activity of the enzyme is generally considered to be essential for a normal rate of clearance of triglycerides from the plasma. It is believed that lipoprotein lipase is active mainly in the capillary endothelium. It is not present in plasma except transiently after administration of heparin or similar polyanions (1) and perhaps after dietary fat loads as well (2).

Although the TGL activity in postheparin plasma is often considered to be identical with that of lipoprotein lipase, a number of observations have not been compatible with this hypothesis. The TGL activity extracted from adipose tissue, heart, and certain other tissues is completely inhibited by sodium chloride and protamine sulfate (1, 3). On the other hand, TGL activity in postheparin plasma is variably altered by these substances (4–6). Sodium chloride appears to irreversibly inactivate TGL extracted from rat heart (7), but the effects of sodium chloride and protamine on TGL in plasma are reversible and are dependent on the manner in which the substrate is prepared (4).

Total TGL activity in postheparin plasma also does not always correlate well with plasma triglyceride concentrations. In at least one patient who phenotypically represents type I hyperlipoproteinemia (8) and who is therefore presumably deficient in lipoprotein lipase, postheparin TGL activity has been normal when measured by a conventional assay (5). Conversely, subjects receiving estrogens may have activity well below normal yet have normal plasma triglyceride concentrations (9).

Experiments undertaken to determine possible heterogeneity in TGL activity are described in this report. They indicate that heparin releases or activates from liver a TGL activity that has different properties from the TGL obtained from adipose and other tissues. Postheparin plasma contains TGL activity with properties that resemble most closely those of the TGL from liver.

MATERIALS AND METHODS

Standard triglyceride lipase assay

The standard assay was carried out by a modification of a previously described system (6). Substrates were prepared by first adding 14 nmoles of [1-^{14}C]trioleate (5.5 × 10^5 dpm, Nuclear-Chicago) in benzene to a 20-ml counting vial. Benzene was removed under nitrogen. 0.01 M Tris–HCl buffer, pH 8.6, in 0.15 M NaCl (referred to as

TABLE 1. Ingredients of TGL assay systems

A	B	C
14 nmoles [^{14}C]triolein in benzene	14 nmoles [^{14}C]triolein in benzene	14 nmoles [^{14}C]triolein in benzene 125 nmoles unlabeled triolein
benzene evaporated	benzene evaporated	benzene evaporated
6.0 ml standard buffer 0.2 ml 1% albumin 0.1 ml 1:100 Triton X-100 sonication	6.0 ml standard buffer 0.2 ml 1% albumin 0.1 ml 1:100 Triton X-100 0.1 ml heparin solution, 100 units/ml 0.2 ml preheparin plasma^a sonication	6.0 ml standard buffer 0.2 ml 1% albumin 0.1 ml 1:100 Triton X-100 0.1 ml heparin solution, 100 units/ml 0.4 ml varying amts of HDL solution (20 mg HDL protein/ml) or standard buffer (see Fig. 5) sonication
↙ ↘ 3.0-ml aliquot 3.0-ml aliquot + 0.15 ml human or rat postheparin plasma or rat liver perfusate	↙ ↘ 3.0-ml aliquot 3.0-ml aliquot + 0.5 ml NH$_4$OH–NH$_4$Cl or Krebs-Ringer extract ^{a} plasma triglyceride = 80 mg/100 ml; plasma HDL = 50 mg cholesterol/100 ml (approx 1.25 mg protein/ml)	↙ ↘ 3.0-ml aliquot 3.0-ml aliquot + 0.15 ml rat liver perfusate or 0.5 ml NH$_4$OH–NH$_4$Cl tissue extract

D	E	F
1.0 ml 1:10 dilution^b of Ediol 2.0 ml 20% albumin in 0.1 M NH$_4$SO$_4$, pH 8.4 0.5 ml heparin solution, 100 units/ml 1.0 ml preheparin plasma 1.0 ml NH$_4$OH–NH$_4$Cl tissue extract ^{b} containing 5 mg of triglyceride/ml	3.0 ml 0.1 M NH$_4$OH–NH$_4$Cl buffer containing 0.3 ml 10% Intralipid^c 0.5 ml heparin solution, 100 units/ml 1.0 ml preheparin plasma 1.0 ml NH$_4$OH–NH$_4$Cl tissue extract ^{c} containing 10 mg of triglyceride/ml	1.0 ml VLDL or chylomicron preparation^d 1.0 ml 20% albumin 0.5 ml heparin solution, 100 units/ml 2.0 ml NH$_4$OH–NH$_4$Cl tissue extract ^{d} containing 250 mg of triglyceride/ml

standard buffer), a 1% albumin solution, and a 1:100 dilution of Triton X-100 solution were mixed in the counting vial in the proportions summarized in Table 1, *A*. This mixture was sonicated for 1 min with a Branson Sonifier, and 3.0 ml was pipetted into each of two duplicate counting vials. To each vial was added 0.15 ml of postheparin plasma or liver perfusate, which served as a source of enzyme, unlabeled triglyceride, and HDL cofactor.

Unless otherwise stated, all incubations were carried out in a Dubnoff shaking water bath at 27°C for 1 hr. In some experiments, aliquots were removed at 15–20-min intervals for determination of ^{14}C-labeled or total fatty acids.

After incubation, fatty acids were extracted from 1.0 ml of the incubate by the method of Dole and Meinertz (10) and isolated on ion-exchange resin by the method of Kelley (11). All results were calculated in terms of nmoles of labeled fatty acid liberated per milliliter of enzyme source per hour. With this assay system, plasma enzyme activity has been demonstrated to increase linearly with time, as shown by Greten, Levy, and Fredrickson (6). Under similar conditions of assay (described below), enzyme activity proceeded linearly when liver extracts or perfusates or adipose tissue and heart extracts were used as enzyme source. Although concentrations of substrate were suboptimal, the assay method was suitable for comparative studies of the effects of inhibitors of enzyme activity. In addition, enzyme activity in-

creased in a linear fashion when enzyme concentration was increased over the range used in these experiments.[1]

Modifications of the standard TGL assay

In some experiments, the assay system was modified to differentiate between TGL activities.

Ionic strength was varied by diluting 0.01 M Tris–HCl, pH 8.6, with 0.01 M Tris–HCl (pH 8.6) in 5 M NaCl to a total volume of 6.0 ml and substituting this for standard buffer (see Table 1, *A* and *B*). Protamine sulfate was added by mixing 0.6 ml of 0.1 M Tris–HCl, pH 8.6, containing 0.15 M NaCl and 3.0 mg/ml of protamine sulfate, with 5.4 ml of standard buffer. The final concentration of protamine sulfate was 300 µg/ml. Sodium pyrophosphate was added to a final concentration of 10 nmoles/ml by addition of 0.6 ml of 0.1 mM sodium pyrophosphate (in 0.01 M Tris, 0.15 M NaCl) to 5.4 ml of standard buffer. In some studies, the pH of the standard buffer was adjusted with either NaOH or HCl (and these solutions were then substituted for the standard buffer).

In experiments in which NH$_4$OH–NH$_4$Cl extracts of acetone–ether powders or Krebs-Ringer extracts were used as enzyme sources (see below), sodium heparin and preheparin plasma were added before sonication (Table 1, *B*). When HDL was evaluated for its ability to enhance enzyme activity, unlabeled triolein (Hormel

---

[1] LaRosa, J. C. Unpublished observations.

Institute) and different amounts of HDL were added in place of preheparin plasma (Table 1, C).

Substrates other than [¹⁴C]triolein were used in some experiments to assay tissue extracts. These included artificial triglyceride emulsions and lipoproteins. The former were either Ediol (Riker Laboratories, Northridge, Calif.) or Intralipid (10%, Vitrum, Stockholm, Sweden). In a modification of a previously reported assay system (5), Ediol was incubated with albumin, heparin, preheparin plasma (added as a source of HDL cofactor), and tissue extracts (Table 1, D). Intralipid was prepared in 0.1 M NH₄OH–NH₄Cl buffer containing 10% albumin, as described by Boberg and Carlson (12), and incubated with heparin, preheparin plasma, and tissue extracts (Table 1, E).

Chylomicrons were prepared (13) from the plasma of two patients with type I hyperlipoproteinemia who were eating ad lib. VLDL were obtained (14) from a patient with type IV hyperlipoproteinemia whose diet for the preceding week had contained only 5 g of fat per day. Triglycerides were determined by the method of Kessler and Lederer (15). The lipoproteins were incubated with heparin, albumin, and tissue extract (Table 1, F). Increments in fatty acid concentration were determined by the method of Dole and Meinertz (10).

Sources of enzyme activity

Postheparin plasma. Human postheparin plasma was collected from normal subjects 10 min after intravenous administration of sodium heparin, 10 units/kg of body weight. The blood was placed in tubes containing heparin (1 unit/ml) as anticoagulant (5). Rat plasma was obtained from NIH Osborne-Mendel rats (250–300 g) maintained on standard chow ad lib. The rats were anesthetized with ether and given 100 units of heparin in a femoral vein. 10 min after injection, the animals were killed by exsanguination from the aorta; the blood samples were immediately centrifuged at 4°C, and the plasma was decanted. Plasma samples were then either stored at −20°C or used immediately (see Table 1, A).

Tissue triglyceride lipases. TGL activity was obtained from rat tissues by four different methods.

Acetone–ether powders of heart, lung, kidney, spleen, liver, adipose tissue, and intestine were prepared as previously described (6). They were either stored in vacuo at 4°C for up to 72 hr or were used immediately. 5 mg of powder was homogenized in 1.0 ml of 0.025 M NH₄OH–NH₄Cl, pH 8.6, in a Potter-Elvehjem homogenizer for 2–4 min at 4°C. The suspension was centrifuged, and aliquots of the supernatant layer were used as enzyme source (see Table 1, B–F).

Adipose tissue was also extracted directly by homogenizing 20 g (wet wt) of tissue in 20 ml of the NH₄OH–NH₄Cl buffer for 5 min in a Sorvall Omni-Mixer at 0°C.

The suspension was centrifuged, the fat layer was removed from the top of the tube, and aliquots of the infranatant layer were used as enzyme source (Table 1, B).

Rat epididymal fat pads or liver slices were placed in Krebs-Ringer phosphate buffer, 100 mg wet wt of tissue/ml, and incubated at 37°C. The buffer contained sodium heparin, 0.2 unit/ml; it was previously determined that this increased the yield of lipase 3–4-fold. The incubation medium was decanted at the end of 1 hr, and aliquots were used as a source of enzyme. In these assays, additional heparin was not added to the medium (Table 1, B).

Lipase was also obtained in heparin-containing perfusates of rat liver (16). The donor rats were males weighing approximately 300 g; they had been fed standard chow ad lib. The perfusate was from rat blood, which was immediately defibrinated by stirring with a glass rod, cooled to 4°C, and diluted with an equal volume of Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin (fraction V) and 0.1% glucose and adjusted to pH 7.4 with Na₂CO₃. The mixture (hematocrit of 22%) was filtered through a column of glass wool. It was then warmed to 37°C, and a 110-ml portion was transferred to each oxygenator–reservoir of the perfusion apparatus where it was continuously gassed with a humidified O₂–CO₂ mixture (95:5, v/v). The livers were perfused at a constant rate of 20.4 ml/min. For the first 90 sec of perfusion, effluent from the livers was collected and discarded. Thereafter, the perfusate was recycled back to the oxygenator–reservoir. The recycling volume was therefore 80 ml. The perfusate was sampled (10 ml) 30 min after the start of perfusion. Then, 1 ml of 0.9% NaCl containing 10,000 units of heparin sodium was added to the perfusate reservoir. In control perfusions, the NaCl solutions contained no heparin. The perfusion was continued for 30 additional min, and then the perfusate was sampled again. Perfusate samples were immediately chilled in ice and centrifuged to remove the cells. The plasma was virtually free of hemoglobin, and 0.15-ml samples were assayed for lipase activity. The livers maintained a normal appearance throughout the period of perfusion.

Mixing of adipose tissue and plasma

Equal volumes of an NH₄OH–NH₄Cl extract of acetone–ether powder from rat adipose tissue and either pre- or postheparin rat plasma were incubated for 30 min at 4°C, and 0.3-ml aliquots of this mixture were used as enzyme (see Table 1, B).

Preincubation with NaCl

In certain experiments, enzyme extracts were incubated with NaCl before assay. The NH₄OH–NH₄Cl extracts of acetone–ether powder of liver and adipose

tissue were mixed with an equal volume of a solution containing 2 M NaCl in 0.01 M Tris–HCl, pH 8.6, and shaken gently at 4°C for 30 min. 0.5 ml was added to substrate prepared in 0.01 M Tris (Table 1, *B*). The final concentration of NaCl was approximately 0.15 M.

Plasma or liver perfusate samples were similarly incubated with an equal volume of the NaCl solution. 0.15 ml of the mixture was added to substrate prepared in the standard buffer (Table 1, *A*) such that the final salt concentration was approximately 0.2 M NaCl.

Preparation of HDL

Plasma HDL (d 1.063–1.21) was obtained as previously reported (14) from three normal subjects. The preparations were examined immunochemically with appropriate antisera (17) for the presence of albumin, LDL, and other plasma protein contaminants. Except for trace amounts of LDL in some preparations, they appeared to be pure. HDL protein determinations were performed by the method of Lowry et al. (18).

Preheparin plasma. Two units of plasma were obtained in standard citrate blood packs (Fenwall Laboratories, Morton Grove, Ill.) from a normal female donor who had fasted overnight. Triglyceride levels were 80 mg/100 ml and HDL (expressed as α-lipoprotein cholesterol [8]) was 50 mg/100 ml. The plasma was kept frozen in small aliquots for use as a source of unlabeled triglyceride and HDL cofactor. The same plasma was used in all experiments calling for preheparin plasma (see Table 1). No TGL activity was detected in this plasma.

RESULTS

Effects of inhibitors on TGL from different sources

TGL activity from postheparin plasma, adipose tissue, and liver was compared with respect to the influence of three inhibitors of lipoprotein lipase: NaCl, protamine sulfate, and pyrophosphate.

Postheparin plasma. TGL activity in human and rat postheparin plasma differed considerably in its response to these inhibitors. The effect of increasing ionic strength by increments in NaCl added to the incubation mixture is illustrated in Fig. 1. Data from plasma samples taken from nine normal human subjects are presented as well as those from plasma samples from two rats. Two peaks of TGL activity were obtained when ionic strength was changed (conductivity from approximately 0 to 100 mmhos). The initial peak was obtained at approximately 9 mmhos (0.15 M NaCl). The activity in both human and rat samples was 40% less at 25 mmhos (0.5 M NaCl), and again increased at 45–75 mmhos (1.0–2.0 M NaCl).

The effect of adding protamine sulfate or pyrophosphate is also illustrated in Fig. 1. Data from five human
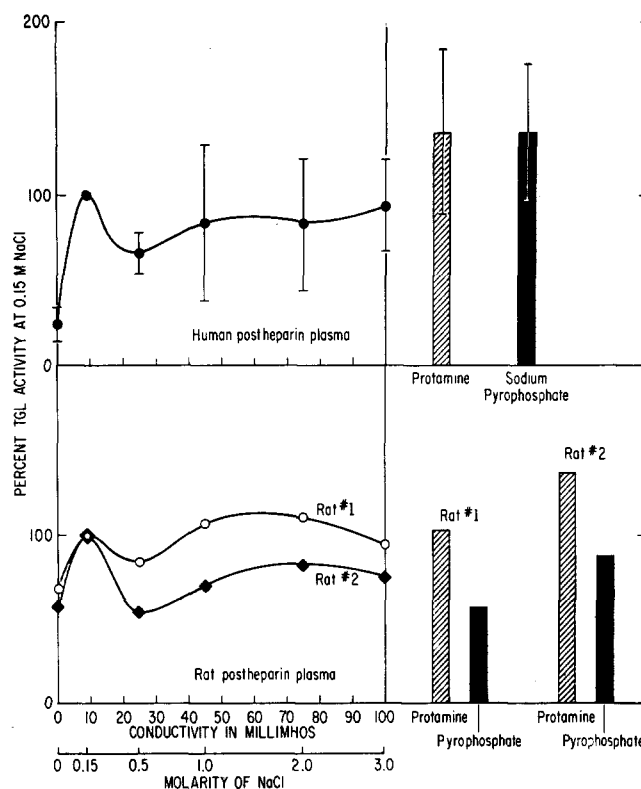

FIG. 1. Effect of inhibitors on plasma postheparin TGL. On the left, the effect of increasing ionic strength is depicted. Data from the postheparin plasma of nine normal human subjects (mean ± SD) and of two rats are shown. On the right, the effects of protamine (300 μg/ml) and pyrophosphate (10 nmoles/ml) on the same plasma samples are shown. The data represent five human subjects (means and SD) and two rats. For ease of comparison and because absolute levels of TGL varied markedly, results are plotted as percentage of activity in 0.15 M NaCl (conductivity = 9 mmhos).

plasma samples are represented; *no* appreciable inhibition by protamine or pyrophosphate was observed. Activity in plasma from both rats was decreased by the addition of pyrophosphate. All of these incubations were carried out at constant ionic strength in 0.15 M NaCl. Postheparin samples that were not inhibited by the concentrations of protamine or pyrophosphate shown in Fig. 1 also were not inhibited when these concentrations were increased tenfold.

Postheparin plasma was also extracted with petroleum ether–diethyl ether 9:1 (6) in a manner similar to that used to obtain lipid-free enzyme from adipose tissue. The extracted residue contained approximately 50% of the TGL activity of the starting plasma; the response of this activity to changing ionic strength or to addition of protamine or pyrophosphate was similar to that of unextracted plasma.

Adipose tissue TGL. TGL activity in adipose tissue was reproducibly decreased when either NaCl, protamine, or pyrophosphate was added to the incubation. In Fig. 2 are shown the activities obtained in NaCl at
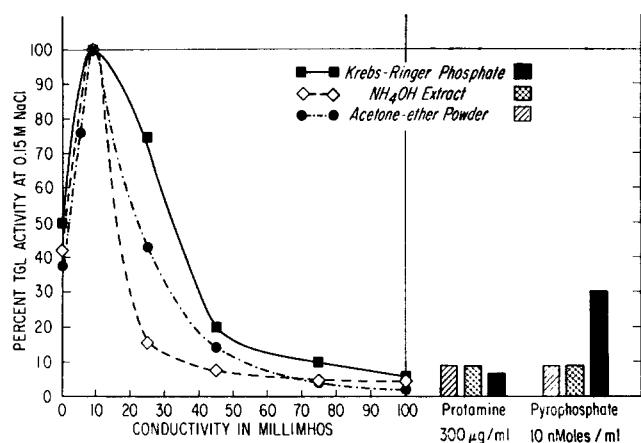
Fig. 2. Effect of inhibitors on adipose tissue TGL. For purposes of comparison, all data are expressed as percentage of activity at 0.15 M NaCl (9 mmhos).

various ionic strengths. Essentially the same results were obtained with enzyme activity derived from five different acetone–ether powders as well as two obtained by extraction with NH_4OH–NH_4Cl and two obtained by incubation of adipose tissue in Krebs-Ringer–heparin solution. Only one maximum of activity appeared, at 9 mmhos (0.15 M NaCl). At a conductivity of 45 mmhos (1.0 M NaCl), activity was only 10% of the maximum and declined further with increasing ionic strengths.

Each of the enzyme preparations obtained by acetone–ether or NH_4OH–NH_4Cl extraction was inhibited more than 90% by either protamine (300 μg/ml) or pyrophosphate (10 nmoles/ml). The enzyme extracted in Krebs-Ringer phosphate buffer was inhibited approximately 70%.

TGL from rat liver and other tissues. In Fig. 3 are illustrated the effects of inhibitors on TGL obtained from
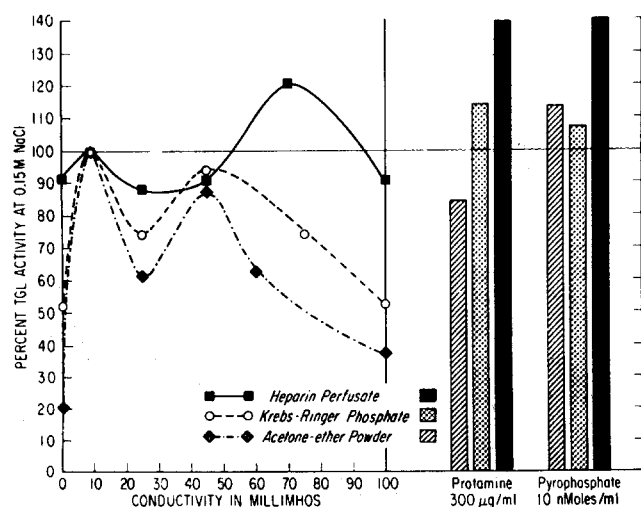
rat liver by perfusion, by incubation of the whole tissue in Krebs-Ringer phosphate, or by acetone–ether extraction. Two liver perfusates and several of each of the other preparations were tested; the results were qualitatively the same. Liver TGL activity responded similarly to postheparin plasma. There was a bimodal curve of activity as ionic strength was increased. There also was no convincing inhibition by either protamine or pyrophosphate. Lipase activity from other rat tissues was also examined. Rat heart, lung, kidney, spleen, and intestine all contained heparin-extractable TGL activity with properties identical with those of adipose tissue triglyceride lipase (Fig. 4). Two rat livers that were perfused without heparin released essentially no TGL activity, but substantial activity was released from livers perfused with heparin.

Further comparisons of TGL from adipose tissue and liver

Effect of HDL. One of the most important criteria employed for the identification of lipoprotein lipase has been the requirement for HDL as a cofactor (7). In two experiments, adipose tissue TGL activity was markedly enhanced by the presence of HDL in the incubation media (Fig. 5). Activity progressively increased with each increment of HDL. The final HDL concentration tested was roughly half that in normal human plasma. TGL activity from liver, on the other hand, was inhibited by increasing the quantity of HDL in the medium (Fig. 5).

Hydrolysis of different triglyceride substrates. In one experiment, acetone–ether powders of liver and adipose tissue TGL having equivalent activity against [14C]-triolein were incubated with four other substrates. The

Fig. 3. Effect of inhibitors on rat liver TGL. All data are presented as the percentage of activity obtained at 0.15 M NaCl (9 mmhos).
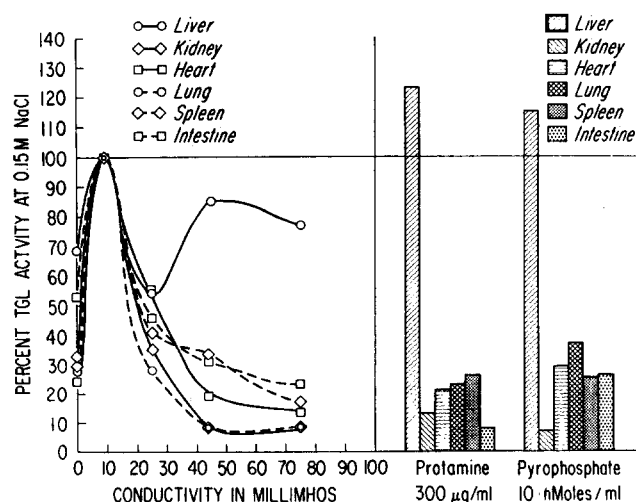

Fig. 4. Effect of inhibitors on TGL from six different rat tissues. All data are presented as the percentage of activity at 0.15 M NaCl.
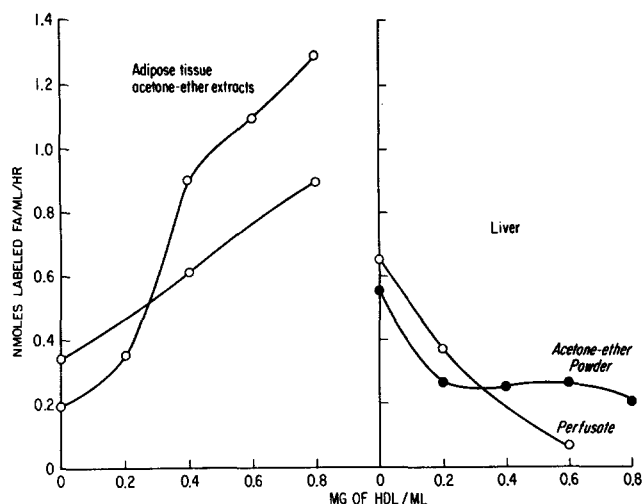
FIG. 5. Effect of HDL on lipase activity in adipose tissue and liver. The incubation mixture contained no plasma. Adipose tissue activity was obtained from extracts of acetone–ether powders; rat liver TGL was obtained from both acetone–ether powders of whole rat liver and a heparin perfusion of rat liver. FA = fatty acid.

FIG. 7. TGL activity after preincubation of postheparin plasma, liver perfusate, and adipose tissue extracts with NaCl. Only adipose tissue is significantly altered by preincubation with NaCl. FA = fatty acid.

preparations were equally active against the artificial substrates, Intralipid and Ediol. Only adipose tissue TGL promoted significantly the hydrolysis of triglycerides in chylomicrons or VLDL (Fig. 6).

Preincubation with NaCl. Preincubation of adipose tissue with NaCl inhibited lipase activity as effectively as the addition of NaCl to the assay medium. The TGL activities in liver or postheparin plasma, however, were not inhibited by NaCl (Fig. 7). Results obtained with liver perfusate are shown. Similar data were obtained with NH_4OH–NH_4Cl and Krebs-Ringer extracts of rat liver.
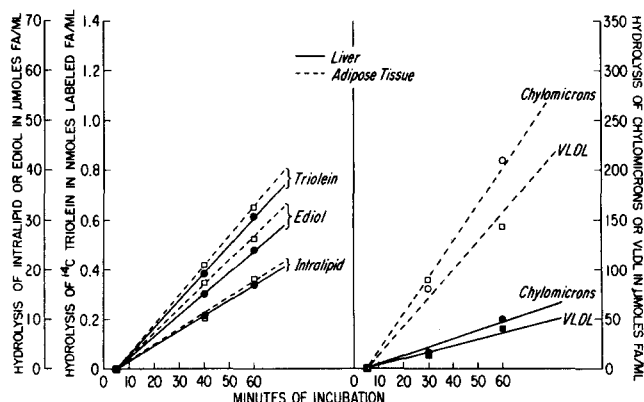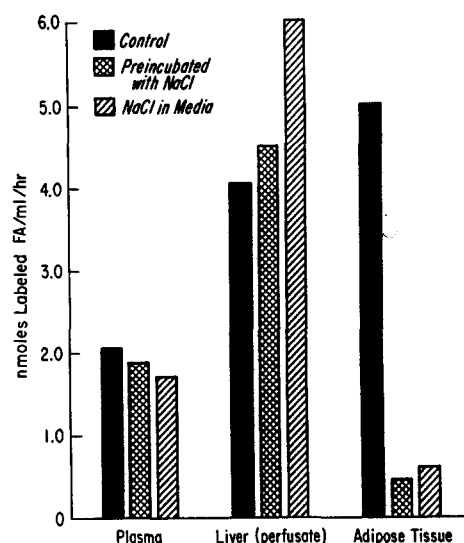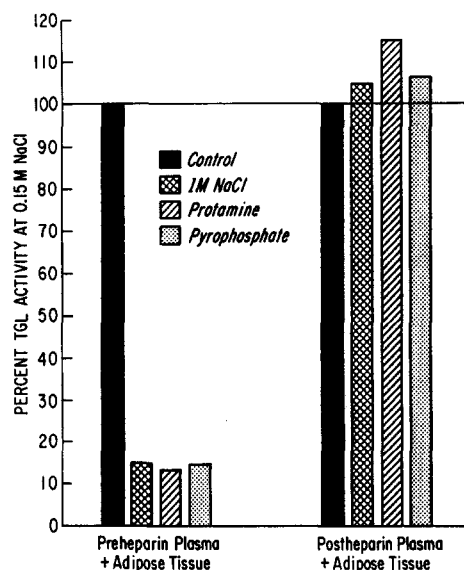

FIG. 6. Relative hydrolysis of different substrates by adipose tissue and liver. On the left are depicted hydrolysis rates of the same *acetone–ether extractions* of adipose tissue and liver against [^{14}C]triolein, Ediol, and Intralipid. On the right, the results of incubation with chylomicrons and VLDL are shown. FA = fatty acid.

FIG. 8. Mixing of adipose tissue and pre- and postheparin plasma. For comparison, data are presented as percentage o control. The conductivity of the medium was identical in the control, protamine, and pyrophosphate incubations.

Liver TGL activity was completely inactivated by incubation at 37°C for 75 min. This is comparable to TGL activity in both postheparin plasma and adipose tissue.

Mixing of adipose tissue and plasma. The properties of adipose tissue and TGL in postheparin plasma were not altered by the mixing experiment shown in Fig. 8. There appears to be no transferable factor in plasma or adipose tissue which alters the properties of TGL from either.

DISCUSSION

These studies confirm and considerably extend earlier observations that the properties of TGL activity in adipose tissue and postheparin plasma are not identical (4, 5). Adipose tissue TGL activity is completely inhibited by NaCl, sodium pyrophosphate, and protamine added to the incubation mixture, and by preincubation in solutions of high ionic strength. Plasma TGL activity is not completely inhibited and may be enhanced under these same conditions. In its response to alterations in ionic strength and to other inhibitors, TGL activity in postheparin plasma resembles TGL activity present in liver. Other workers have detected triglyceride lipase activity in liver (19–25). Whether this activity is identical with that of lipoprotein lipase remains conjectural. Some investigators (7, 21–23) have observed up to 50% inhibition of hepatic lipase with 1 M NaCl or protamine sulfate. Spitzer and Spitzer (24), on the other hand, have reported enhancement of lipase activity from rat liver perfusions in the presence of protamine. The present experiments have further defined the effect of NaCl on liver TGL activity with the demonstration of enhancement of activity at higher ionic strengths. Other differences between hepatic lipase and tissue "lipoprotein lipase" have also been demonstrated. HDL, which activates adipose tissue TGL, has been found to inhibit liver TGL activity. Liver TGL, moreover, has been shown to be ineffective in catalyzing hydrolysis of triglycerides in lipoproteins as opposed to those in artificial emulsions. During the course of our work, experiments by Hamilton (25) were brought to our attention. He also found that heparin perfusates of dog liver catalyzed the hydrolysis of artificial triglyceride emulsions but not of chylomicrons. This TGL activity was resistant to protamine.

It might be inferred from the present experiments that postheparin plasma contains no TGL with the properties of the adipose tissue enzyme. There are data, however, that suggest that heparin causes release of TGL activity into plasma from heart and adipose tissue (26) as well as from the liver. Moreover, by adding large amounts of HDL to the incubation mixture, one can demonstrate that some of the TGL present in postheparin plasma undergoes alteration in its properties. Its activity at low ionic strengths increases, and it becomes more susceptible to inhibition by high ionic strengths, protamine sulfate, or sodium pyrophosphate. These findings have been reported in preliminary form (27).

Other workers have concluded that there are no differences between TGL activity in adipose tissue and in postheparin plasma. Boberg and Carlson (12), using Intralipid as substrate, have found TGL activity in postheparin plasma to be reproducibly and completely inhibited by salt. Datta and Wiggins (4) have observed that the activity in postheparin plasma is inhibited more by salt when the assay employs triglyceride emulsions stabilized with lecithin, such as Intralipid, than when the substrate is an emulsion prepared by sonication and stabilized by nonionic detergents, as was the triolein used in our studies. As originally suggested by Korn (7), differences in responses of lipases to salt that are related to the substrate or other assay conditions per se might be circumvented by preincubating the enzyme in a solution of high ionic strength and then performing the assay at physiological ionic strength. Korn (7) found that TGL activity in rat heart is inactivated whether it is preincubated with salt or assayed with salt in the media. We have shown this to be true of adipose tissue TGL and have observed that TGL in postheparin plasma and rat liver perfusates are not inhibited under either condition.

A more intriguing and unresolved question is whether these and similar studies are concerned with more than one triglyceride lipase or a single enzyme whose properties are variably altered by methods of assay and preparation and other possible factors. Some crude attempts have been made to determine whether methods of preparation might induce different properties to TGL activity obtained from different sources. Extraction of adipose tissue or liver by acetone–ether or of plasma by petroleum ether–diethyl ether (6) does not alter the effects of inhibitors on the TGL activity obtained. The cross-incubation experiments described in Fig. 8 also indicated that no readily transferable factors in plasma or adipose tissue can influence the properties of the TGL activity regularly found in these tissues. These experiments, of course, do not exclude transformation of the same enzyme to different forms either within tissues or upon extraction. Such transformations could conceivably be related, for example, to the availability of heparin or other polyanions. Mayes and Felts (28), for example, have reported that TGL in rat liver homogenates were more sensitive to the inhibitory action of sodium chloride when additional heparin was added to the assay system. Nevertheless, these studies raise the possibility that TGL in postheparin plasma may be heterogeneous and that its measurement may not accurately reflect the capacity for removal of triglyceride from the plasma.

REFERENCES

1. Korn, E. D. 1959. The assay of lipoprotein lipase in vivo and in vitro. *Methods Biochem. Anal.* **7:** 145–192.
2. Sailer, S., F. Sandhofer, and H. Braunsteiner. 1966. Regulation of endogenous lipoprotein-lipase activity in the

plasma of normal subjects and patients with essential hyper-lipaemia. *Ger. Med. Mon.* **11:** 41–43.

3. Korn, E. D., and T. W. Quigley. 1957. Lipoprotein lipase of chicken adipose tissue. *J. Biol. Chem.* **226:** 833–839.

4. Datta, D. V., and H. S. Wiggins. 1964. New effects of sodium chloride and protamine on human post-heparin plasma "lipoprotein lipase" activity. *Proc. Soc. Exp. Biol. Med.* **115:** 788–792.

5. Fredrickson, D. S., K. Ono, and L. L. Davis. 1963. Lipo-lytic activity of post-heparin plasma in hyperglyceri-demia. *J. Lipid Res.* **4:** 24–33.

6. Greten, H., R. I. Levy, and D. S. Fredrickson. 1968. A further characterization of lipoprotein lipase. *Biochim. Biophys. Acta.* **164:** 185–194.

7. Korn, E. D. 1955. Clearing factor, a heparin activated lipoprotein lipase. *J. Biol. Chem.* **215:** 1–14.

8. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276:** 34–44, 94–103, 148–156, 215–225, 273–281.

9. Hazzard, W. R., M. J. Spiger, J. D. Bagdade, and E. L. Bierman. 1969. Studies on the mechanism of increased plasma triglyceride levels induced by oral contraceptives. *N. Engl. J. Med.* **280:** 471–474.

10. Dole, V. P., and H. Meinertz. 1960. Microdetermination of long-chain fatty acids in plasma and tissues. *J. Biol. Chem.* **235:** 2595–2599.

11. Kelley, T. F. 1968. Rapid assay of labeled free fatty acids in mixtures of labeled lipids. *J. Lipid Res.* **9:** 799–800.

12. Boberg, J., and L. A. Carlson. 1964. Determination of heparin induced lipoprotein lipase activity in human plasma. *Clin. Chim. Acta.* **10:** 420–427.

13. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentri-fugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.

14. LaRosa, J. C., R. I. Levy, W. V. Brown, and D. S. Fred-rickson. 1971. Changes in high density lipoprotein protein composition after heparin-induced lipolysis. *Amer. J. Physiol.* **220:** 785–791.

15. Kessler, G., and H. Lederer. 1965. Fluorometric meas-urement of triglyceride. *In* Automation in Analytical Chemistry. L. T. Skeggs, Jr., editor. Mediad Inc., New York. 341–344.

16. Windmueller, H. G., and A. E. Spaeth. 1966. Perfusion in situ with tritium oxide to measure hepatic lipogenesis and lipid secretion. *J. Biol. Chem.* **241:** 2891–2899.

17. Levy, R. I., R. S. Lees, and D. S. Fredrickson. 1966. The nature of pre-beta (very low density) lipoproteins. *J. Clin. Invest.* **45:** 63–77.

18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

19. Olson, A. C., and P. Alaupovic. 1966. Rat liver triglyceride lipase. *Biochim. Biophys. Acta.* **125:** 185–187.

20. Guder, W., L. Weiss, and O. Wieland. 1969. Triglyceride breakdown in rat liver: the demonstration of three differ-ent lipases. *Biochim. Biophys. Acta.* **187:** 173–185.

21. Felts, J. M., and P. A. Mayes. 1967. Release of lipoprotein lipase from the perfused liver of the rat. *Nature* (London). **214:** 620–621.

22. Boberg, J., L. A. Carlson, and L. Normell. 1964. Produc-tion of lipolytic activity by the isolated, perfused dog liver in response to heparin. *Life Sci.* **3:** 1011–1019.

23. Muir, J. R. 1968. The regional production of lipoprotein lipase in man. *Clin. Sci.* **34:** 261–270.

24. Spitzer, J. A., and J. J. Spitzer. 1956. Effect of liver on lipolysis by normal and post-heparin sera in the rat. *Amer. J. Physiol.* **185:** 18–22.

25. Hamilton, R. L. 1965. Post-heparin plasma lipase from the hepatic circulation. *Diss. Abstr.* **26:** 24.

26. Payza, A. N., H. B. Eiber, and S. Walters. 1967. Studies with clearing factor. V. State of tissue lipase after injection of heparin. *Proc. Soc. Exp. Biol. Med.* **125:** 188–192.

27. Herbert, P., J. LaRosa, R. Krauss, S. Lux, R. I. Levy, and D. S. Fredrickson. 1971. On the lipolytic defect in type I hyperlipoproteinemia. *J. Clin. Invest.* **50:** 449. (Abstr.)

28. Mayes, P. A., and J. M. Fells. 1968. The functional status of lipoprotein lipase in rat liver. *Biochem. J.* **108:** 483–487.