

Selective measurement of two different triglyceride lipase activities in rat postheparin plasma

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Abstract Conclusive evidence has been obtained for the presence of both hepatic and extrahepatic triglyceride lipase activities (TGLA) in rat postheparin plasma, and an assay has been devised for their selective measurement. Heparin-released TGLA in plasma from the intact rat, like TGLA in postheparin hepatic perfusate, was relatively resistant to inactivation by salt and protamine. Postheparin TGLA obtained from the supradiaphragmatic portion of the rat, where any hepatic contribution was eliminated, was nearly completely inactivated by salt and protamine. Utilizing the different sensitivities to protamine inactivation of extrahepatic and hepatic TGLA, assay conditions were selected to achieve simultaneously the maximal reduction of extrahepatic TGLA with preservation of hepatic TGLA. This assay was validated using postheparin plasma from partially hepatectomized rats. The protamine-inactivated activity was independent of the amount of liver removed, whereas protamine-resistant activity was directly proportional to the amount of liver remaining. In the intact rat, liver appeared to be the major source of heparin-released TGLA measured at pH 8.6 with triolein substrate. It was further shown that both hepatic and extrahepatic lipases catalyzed hydrolysis of triglyceride-rich lipoproteins.

Supplementary key words lipoprotein lipase · hepatic triglyceride lipase · extrahepatic triglyceride lipase · perfused liver · hepatectomy · supradiaphragmatic portion of the rat

LIPOPROTEIN LIPASE is a tissue-bound TGLA (glycerol-ester hydrolase, EC 3.1.1.3) that catalyzes the hydrolysis

of triglyceride in plasma lipoproteins or artificial lipid emulsions "activated" with lipoprotein (1, 2). Initially described in rat heart (1, 2), this enzymatic activity has been subsequently identified in other animal tissues, including adipose tissue (3), diaphragm (4), lung (5), and mammary gland (6, 7). Further studies have suggested that lipoprotein lipase is an important determinant of lipoprotein triglyceride uptake and hydrolysis in rat heart (8) and adipose tissue (9).

The intravenous administration of heparin is known to release TGLA into plasma. While this activity has been shown to share some characteristics with tissue lipoprotein lipase (1, 2), discrepancies have been noted, particularly with regard to the response of postheparin plasma TGLA to inhibitors of lipoprotein lipase (10–13). In a recent report from this laboratory (13), TGLA obtained from rat liver was found to exhibit certain similarities to the TGLA in postheparin plasma, among them a resistance to salt, protamine, and pyrophosphate, which are potent inhibitors of TGLA derived from extrahepatic tissues (1, 13). It was suggested that the release of TGLA from the liver into plasma after heparin injection might account for many of the observed characteristics of TGLA in postheparin plasma. These studies, however, provided no direct evidence for separate contributions of hepatic and extrahepatic tissues to plasma TGLA. Furthermore, no means was afforded for the separate quantitation of either the hepatic or the extrahepatic component.

The current studies were designed to evaluate several issues raised by these previous investigations. We first obtained evidence for the presence of hepatic TGLA in rat postheparin plasma. We were then able to devise an assay for the separate measurement of hepatic and extrahepatic TGLA and to quantify their contributions

Abbreviations: TGLA, triglyceride lipase activity; SD, supradiaphragmatic; VLDL, very low density lipoprotein; FFA, free fatty acid.

to the total TGLA ordinarily measured in postheparin plasma at pH 8.6. Finally, we compared the ability of hepatic and extrahepatic lipase to hydrolyze triglyceride in very low density lipoproteins.

METHODS

Enzyme sources

Rat postheparin plasma. NIH Osborne-Mendel male rats weighing 200–400 g, and fed standard chow ad lib., were used for all studies. All operations and blood sampling were performed under ether anesthesia. Blood was obtained by aortic puncture and transferred to tubes containing heparin (130 U/mg; Upjohn Co., Kalamazoo, Mich.), 2 U/ml of blood. Postheparin blood samples were collected exactly 10 min after injection of heparin, 250 U/kg, into the saphenous vein. Plasma was separated in a refrigerated centrifuge.

Partial hepatectomy was performed immediately before heparin administration. The duration of this procedure was between 5 and 10 min. All or portions of the median, left, and right lobes were ligated and excised, and circulation through the remaining liver was left intact. At the end of each experiment the portion of liver remaining in the animal was weighed.

Total hepatectomy was achieved by a modification of the SD preparation of Bezman-Tarcher and Robinson (14). Briefly, rats were anesthetized with a regulated mixture of diethyl ether and oxygen, and loose ligatures were placed around the inferior vena cava between the liver and diaphragm and around the aorta, just cranial to the branching of the celiac artery. A polyethylene catheter (0.023 inch I.D., 0.038 inch O.D.) was inserted into the inferior vena cava at the level of the left renal vein and advanced cranially for 5.5 cm, until the tip lay near the right atrium of the heart. A physiological salt solution containing 40 mg of glucose/ml was infused continuously through this catheter at a rate of 0.013 ml/min. Then both ligatures were tightened simultaneously, thus excluding all infradiaphragmatic tissues from the circulation. The liver, intestines, stomach, spleen, kidneys, and adrenals were excised, and the entire infradiaphragmatic portion of the carcass was removed, care being taken not to cut the polyethylene catheter or the tied end of the aorta. The exposed diaphragm and distal end of the aorta were covered first with a layer of cotton moistened with warmed physiological salt solution and then with a layer of plastic film (Saran Wrap). Then heparin (250 U/kg) was injected via the polyethylene catheter, and after 10 min blood was drawn from the distal end of the aorta.

Perfused rat livers. Isolated rat livers were perfused as previously described (15). The perfusate consisted of

a 1:1 mixture of defibrinated rat blood and Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin (fraction V, Armour Pharmaceutical Co., Chicago, Ill.) and 0.1% glucose and adjusted to pH 7.4 with Na_2CO_3 . The livers were perfused at a rate of 22 ml/min. The first 60 ml passing through the liver was discarded. An additional 50 ml was then perfused through the liver and continuously recycled through a system containing the liver, an oxygenator, and a reservoir. Once recycling was begun, 2500 U of heparin was added (50 U/ml) and perfusion was continued for up to 20 min. No TGLA was present prior to addition of heparin. After heparin was added, the activity was detectable within 2 min, and reached a maximum after 15 min of perfusion. The perfusates used in these studies represented the volume in the reservoir at 15 or 20 min after addition of the heparin.

Postheparin plasma and perfusate plasma were frozen in 1-ml aliquots in dry ice and stored at -10°C ; under these conditions TGLA was stable for at least 6 months.

Rat tissues. Epididymal fat pads and livers were excised from rats after decapitation. Acetone-ether powders were prepared and extracted with 0.025 M NH_4OH by methods described previously (12). The powders were freshly prepared for each assay. Protein concentrations of the extracts were determined by the method of Lowry et al. (16).

Preparation of VLDL

VLDL was isolated by previously described techniques (17) from the plasma of patients with type III and type V hyperlipoproteinemia¹ who had been fed a diet containing no fat for 7 days. Immediately prior to their use, lipoprotein preparations were dialyzed for 16 hr against 0.194 M Tris-HCl buffer of pH 8.6 ($I = 0.05$), containing 0.001 M EDTA and 0.15 M NaCl. Lipoprotein triglyceride concentrations were determined using the method of Kessler and Lederer (18).

Measurement of TGLA

Artificial substrate. The triolein emulsion used as substrate in the basic assay system was prepared by sonicating the following components in a total volume of 12 ml: (1) glyceryl tri[$1\text{-}^{14}\text{C}$]oleate (38 mCi/mmol; Amersham/Searle Corp., Arlington Heights, Ill.), 70 nmol; (2) unlabeled triolein (Applied Science Laboratories, Inc., State College, Pa.), 113 μmol (100 mg); (3) FFA-free bovine serum albumin (Miles Laboratories, Inc., Kankakee, Ill.), 200 mg; (4) Triton X-100 (Packard Instrument Co., Downer's Grove, Ill.), 0.6 ml of a 1% aqueous solution; (5) Tris-HCl buffer, 0.194 M, pH 8.6

¹ VLDL from type V patients were prepared by Dr. P. Herbert.

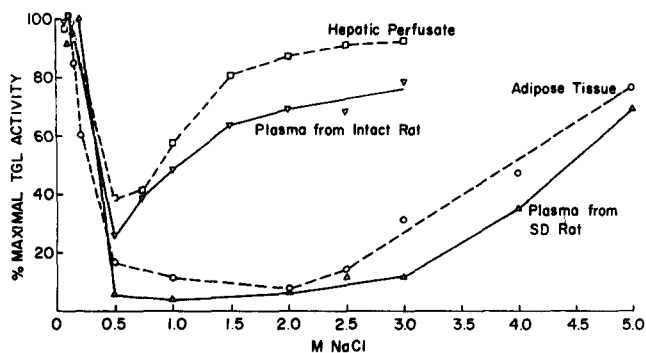


FIG. 1. Effect of preincubation with NaCl on TGLA from different sources. Enzyme sources were preincubated for 60 min at 27°C in a total volume of 0.2 ml with 0.194 M Tris-HCl buffer, pH 8.6 ($I = 0.05$), containing the concentrations of NaCl indicated on the abscissa. After this incubation the NaCl concentration was adjusted to 0.15 M (see Methods), substrate was added, and incubation was continued for 60 min at 27°C. The enzyme sources (one preparation each) were: acetone-ether powder of rat adipose tissue (0.6 mg of protein in 0.05 ml); postheparin hepatic perfusate, 0.1 ml; postheparin plasma from the intact rat, 0.1 ml; and postheparin plasma from the SD rat, 0.1 ml.

($I = 0.05$), containing 0.15 M NaCl. These constituents were sonicated for 1 min at 4°C with a Branson Sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N.Y., model W185) at setting 5.0 (60 W).

Within 30 min of preparation of this emulsion, 0.9 ml (containing 7.5 mg triolein) was mixed with 0.01–0.1 ml of enzyme source (see below) and an appropriate volume of 0.15 M NaCl to make a final incubation volume of 1.0 ml. Heparin and heparinized rat plasma were included in some assays. The mixture was incubated in a Dubnoff shaking water bath at 27°C for 60 min. Extraction of FFA was performed by the procedure of Schotz et al. (19) and ^{14}C -labeled FFA were counted in Instagel (Packard) in a Packard Tri-Carb liquid scintillation spectrometer. Total FFA released was calculated after correction for extraction and counting efficiency, and results were expressed as μmoles of FFA/ml of plasma or mg of protein/hr.

When rat plasma (0.11 ml/ml assay) was added to assays employing rat adipose tissue as enzyme source, a five- to sixfold increase in base line activity could be demonstrated. This "activation" was originally described by Korn for rat heart lipoprotein lipase (2). The addition of plasma or serum did not significantly increase the enzyme activity measured in the assay when the enzyme source itself contained plasma (postheparin plasma or postheparin hepatic perfusate).

In most of these experiments, enzyme sources were preincubated for varying intervals in Tris-HCl buffer containing various concentrations of NaCl or protamine sulfate (salmine, Calbiochem, Los Angeles, Calif.), or both. In every instance, the final NaCl concentration

during assay of enzyme activity was adjusted to 0.15 M. This was accomplished by diluting a 0.05-ml aliquot of the preincubation mixture with 0.05 ml of the Tris-HCl buffer containing sufficient NaCl to achieve an NaCl concentration of 1.5 M. 0.9 ml of the substrate mixture containing no NaCl was then added, bringing the volume to 1 ml and the final NaCl concentration to 0.15 M. This procedure was accomplished within 10 min at 4°C. No inhibitory effect of 1.5 M NaCl was demonstrable under these conditions.

All enzyme incubations were performed in duplicate and deviations between duplicate values averaged less than 5%. In this assay system, hydrolysis of triolein by rat adipose tissue TGLA and hepatic TGLA follows zero-order kinetics for at least 2 hr at 27°C.²

Lipoprotein substrates. VLDL was employed as substrate in an assay system similar to that described above. Triglyceride concentration was adjusted to 8.5 $\mu\text{moles/ml}$, and albumin, salt, and buffer concentrations were the same as used with triolein. The lipoproteins were not sonicated, however, and Triton X-100 was omitted in some assays. Incubations and extractions were carried out as described above, and FFA release was measured by a modification of the method of Dole and Meinertz (20).

RESULTS

Effects of NaCl and protamine on TGLA

It was early shown by Korn (1) that prior incubation of lipoprotein lipase with salt or protamine inhibited the enzyme. We have found that with the present assay system preincubation is necessary because adipose tissue TGLA is protected from inhibition when triglycerides are present in high concentrations in the substrate mixtures.³ In the following experiments, enzyme was incubated with inhibitor for 60 min before addition of the substrate.

TGLA from rat adipose tissue, hepatic perfusate, and postheparin plasma was assayed after preincubation with different concentrations of NaCl (Fig. 1). Maximal activity in all cases was obtained with preincubation in buffer containing 0.1–0.2 M NaCl. Adipose tissue TGLA declined more than 90% as salt concentration was increased to 2.0 M, and then rose progressively with further increase in salt concentration. Similar effects of NaCl on adipose tissue TGLA were obtained when the substrate mixture contained either no heparin, heparin (1.5 U/ml), or heparinized rat plasma (0.11 ml/ml).

² Krauss, R. M. Unpublished data.

³ Krauss, R. M., P. N. Herbert, R. I. Levy, and D. S. Fredrickson. Submitted for publication.

TABLE 1. Protamine inhibition of postheparin TGLA from SD rat in presence of added hepatic perfusate

Enzyme Source ^a	Addition ^a	TGLA		
		-Prot-amine	+Prot-amine	% Inhi-bition
		<i>μmoles FFA/ml/hr</i>		
Postheparin HP	0.15 M NaCl	21.4	6.9	68
Heated postheparin HP ^b	0.15 M NaCl	0.0	0.0	
SD rat plasma	Heated post-heparin HP ^b	21.2	0.3	99

Plasma or perfusates (0.025 ml) were preincubated for 60 min at 27°C with 0.025 ml of heated HP or 0.15 M NaCl in the presence or absence of protamine, 0.15 mg/0.1 ml of preincubation buffer.

^a HP, hepatic perfusate.

^b HP heated at 60°C for 30 min.

Hepatic TGLA was more resistant to the higher NaCl concentrations (Fig. 1). Maximal inhibition was only about 60%, and activity rose steeply as the NaCl concentration was increased above 0.75 M.

The response of postheparin plasma TGLA to increasing salt concentrations paralleled that of hepatic TGLA, although at each level the degree of inhibition was somewhat greater (Fig. 1). The similar behavior of these two activities was consistent with the presence of hepatic TGLA in postheparin plasma. This possibility was examined by measuring the effect of salt on postheparin plasma from the SD rat, from which any hepatic contribution to plasma was excluded. The salt effect differed markedly from that obtained with plasma from intact rats, and corresponded closely to that observed with adipose tissue TGLA (Fig. 1).

These observations were extended by assaying the different TGLA preparations after preincubation with another inhibitor, protamine sulfate (Fig. 2). In all experiments, the concentration of protamine during preincubation was 0.75 mg/ml. The NaCl concentration was varied from 0.1 to 2.5 M, the latter concentration being the maximum in which protamine remained soluble. Adipose tissue TGLA was almost completely inhibited by protamine at all NaCl concentrations. TGLA from hepatic perfusate was more resistant to protamine and, at a concentration of NaCl greater than 0.5 M, appeared to be protected from inhibition. After exposure to protamine at the various salt concentrations, the activity of TGLA in postheparin plasma from the intact rat paralleled that of hepatic TGLA (Fig. 2); as with NaCl alone, however, inhibition of plasma TGLA was somewhat greater at each salt concentration.

In contrast to the enzyme in plasma from the intact rat or hepatic perfusate, TGLA in plasma from the SD rat was nearly completely inhibited by protamine at every salt concentration. This pronounced effect of protamine was not changed by the addition of heat-inacti-

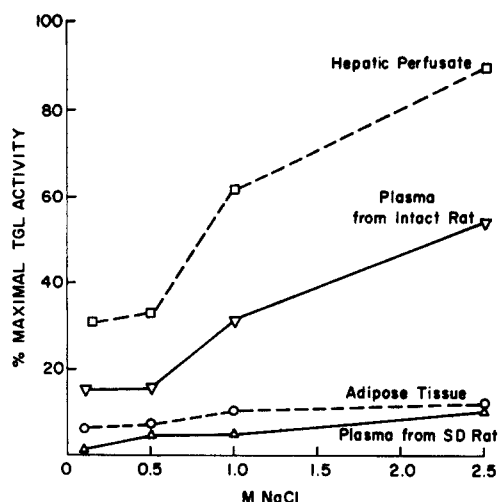


FIG. 2. Effect of preincubation with protamine sulfate on TGLA from different sources. The assay conditions were the same as those described in Fig. 1, except that protamine sulfate, 0.6 mg, was added to the preincubation mixtures. The enzyme sources and key are the same as for Fig. 1. The percent maximal TGLA is relative to the maximal activity obtained for each enzyme in the absence of protamine (100% in Fig. 1).

vated hepatic perfusate to the SD rat plasma (Table 1). It was concluded that hepatic perfusate did not protect heparin-releasable TGLA from inhibition by protamine, but rather contained an enzyme activity which is relatively resistant to inhibition.

The absolute enzyme activities in postheparin plasma from intact and SD rats could be directly compared when measured under optimal assay conditions (100% maximal activity in Fig. 1). The TGLA in plasma from intact rats was 100 ± 12 μ moles of FFA/ml/hr (mean \pm SD, $n = 3$), and in plasma from SD rats the TGLA was 26 ± 9 μ moles of FFA/ml/hr (mean \pm SD, $n = 6$).

Assay conditions for selective measurement of extrahepatic and hepatic TGLA

The foregoing experiments indicated that two different TGLA components were present in postheparin plasma: one of hepatic and the other of extrahepatic origin. These studies did not provide a quantitation of these activities in plasma from the intact rat, but they did suggest that selective measurement might be achieved by taking advantage of their different sensitivities to preincubation with protamine. The most appropriate conditions for this purpose were sought by varying the duration and temperature of incubation and the concentration of protamine. In the initial studies, postheparin plasma from the SD rat and hepatic perfusate were used as sources of extrahepatic and hepatic TGLA, respectively.

Plasma from the SD rat was preincubated at 4°C and 27°C for intervals ranging from 0 to 60 min with

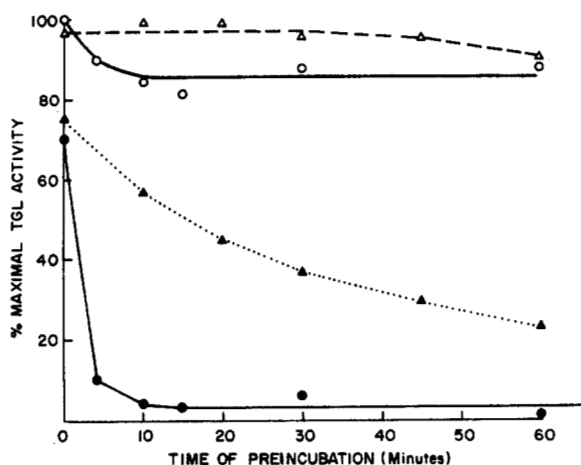


FIG. 3. Effect of time and temperature of preincubation on protamine inhibition of postheparin plasma from the SD rat preparation. 0.1-ml aliquots of postheparin plasma from one SD rat preparation were incubated in the same procedure as described for Fig. 1. The time of preincubation was varied as indicated. Temperature of preincubation and additives to the preincubation buffer were as follows: Δ - Δ , 4°C, 0.1 M NaCl; \blacktriangle ... \blacktriangle , 4°C, 0.1 M NaCl + 0.6 mg protamine; \circ - \circ , 27°C, 0.1 M NaCl; \bullet - \bullet , 27°C, 0.1 M NaCl + 0.6 mg protamine.

protamine sulfate, 1.5 mg/ml (Fig. 3). A 60-min preincubation at 4°C produced a 70% reduction in control enzyme activity. Only 10 min of preincubation at 27°C, however, resulted in a 90% reduction.

When hepatic perfusate was preincubated with protamine at 27°C, the rate of decline of TGLA was much slower (Fig. 4). Activity was reduced by only 10% at 10 min, and 180 min was required to achieve a 90% reduction. At 37°C, the loss of enzyme activity was significantly accelerated (Fig. 4).

From the data in Figs. 3 and 4, it was concluded that simultaneous inactivation of extrahepatic TGLA with preservation of hepatic TGLA could be achieved by prior incubation of enzyme with protamine for 10 min at 27°C. Under these conditions, prior incubation with 3 mg of protamine in a volume of 0.1 ml produced the maximal inactivation of SD rat TGLA and the least inactivation of hepatic TGLA (Fig. 5). In this and the following experiments, rat plasma was included in the preincubation mixture to maintain a plasma or perfusate volume of 0.05 ml.

When 0.5 M NaCl was substituted for protamine in the experiments shown in Figs. 3 and 4, the effects of time and temperature of prior incubation were not significantly altered. Inactivation of either SD rat or hepatic TGLA by protamine or by 0.5 M NaCl was not reversed by dialysis for 16 hr at 4°C against 0.194 M Tris-HCl buffer, pH 8.6, containing 0.15 M NaCl.

Inactivation of TGLA by protamine and salt was also measured after incubation of SD rat plasma and hepatic perfusate with different amounts of heparin (Table 2). Heparin did not influence the inactivation

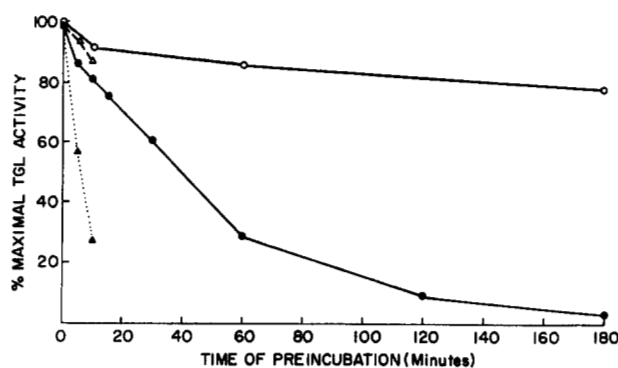


FIG. 4. Effect of time and temperature of preincubation on protamine inhibition of TGLA from postheparin hepatic perfusate. 0.1-ml aliquots of postheparin hepatic perfusate were incubated under the same conditions as described for Fig. 3. Temperature of preincubation and the additives to the preincubation buffer were as follows: \circ - \circ , 27°C, 0.1 M NaCl; \bullet - \bullet , 27°C, 0.1 M NaCl + 0.6 mg protamine; Δ - Δ , 37°C, 0.1 M NaCl; \blacktriangle ... \blacktriangle , 37°C, 0.1 M NaCl + 0.6 mg protamine.

by protamine except at the highest heparin concentration. This quantity of heparin (500 U = 3.8 mg) would probably be sufficient to produce significant ionic binding of the protamine in the assay. Heparin had no effect on the salt inactivation of the TGLA in hepatic perfusate, but in the case of SD rat plasma, there was a marked protection from inactivation which increased as the heparin concentration was raised. In view of possible variations in plasma heparin content, protamine appeared preferable to 0.5 M NaCl as an agent for achieving the selective inactivation of extrahepatic TGLA in postheparin plasma.

Protamine inactivation of TGLA from different sources

The reproducibility of selective inactivation by protamine under the conditions defined above was tested using samples of postheparin plasma from four different SD rats and hepatic perfusate from three different livers (Table 3). The reduction of TGLA in SD rat plasma was $90.6 \pm 1.1\%$ (mean \pm SEM), and the reduction of TGLA in hepatic perfusate was $9.5 \pm 1.4\%$ (mean \pm SEM). These percentages were not altered when the concentration of enzyme from either source was varied over a fivefold range.

TGLA extracted from acetone-ether powders of rat epididymal fat pads or rat livers (0.03 ml) was incubated with heparinized rat plasma (0.05 ml) for 30 min at 37°C in order to simulate the conditions in postheparin plasma. The enzymes were then incubated with protamine as described above. Adipose tissue TGLA was reduced 95% by protamine (from 1.14 to 0.055 μ moles FFA/mg protein/hr), and hepatic tissue TGLA was reduced 10% (from 0.148 to 0.133 μ moles FFA/mg protein/hr). The percentage reduction was the same

when the enzyme concentration was varied by twofold or the heparin concentration by fivefold.

The applicability of this assay to mixtures of TGLA, as may exist in postheparin plasma, was tested by combining SD rat postheparin plasma and hepatic perfusate before preincubation with protamine (Table 4). The TGLA activities in these preparations were additive; the protamine-inactivated activity was equivalent to the SD rat component, and the residual activity was comparable to that in the hepatic perfusate.

Inhibition of TGLA in plasma from hepatectomized rats

A series of rats was subjected to different degrees of partial hepatectomy, ranging from zero (sham-operated) to 80%. Preheparin plasma from these rats was found to contain negligible TGLA. The TGLA in postheparin plasma was assayed after preincubation with 0.1 M NaCl in the presence and absence of protamine. The TGLA present after protamine incubation is referred to as "protamine-resistant" activity, and the difference between control and protamine-resistant TGLA is designated "protamine-inactivated" activity.

The results (Fig. 6) conclusively demonstrated that the protamine-resistant activity decreased in direct proportion to the amount of liver remaining ($r = 0.97$). It was also found that the protamine-inactivated TGLA was independent of the amount of liver tissue removed. Total hepatectomy was not feasible with the surgical technique employed. When TGLA was extrapolated to zero grams of liver (Fig. 6), however, the protamine-resistant activity approached zero, as would be anticipated from results with the SD rat.

TABLE 2. Effect of protamine and 0.5 M NaCl on TGLA from SD rat plasma and hepatic perfusate in the presence of added heparin

Heparin U/0.1 ml	Reduction of TGLA			
	Protamine		0.5 M NaCl	
	SD ^a	HP ^b	SD ^a	HP ^b
				%
0	88.4	9.7	80.3	11.9
1.5	88.8	12.9	61.9	9.6
5	87.8	13.3	29.5	11.1
50	90.4	10.8	16.1	8.8
500	-0.7	-2.1	-2.4	8.5

0.025-ml aliquots of postheparin plasma from one SD rat preparation were incubated with 0.025 ml of heparinized rat plasma and with the indicated quantities of heparin in a total volume of 0.1 ml for 15 min at 27°C. Then, Tris-HCl buffer containing either 3 mg of protamine sulfate or 1.0 M NaCl was added in a volume of 0.1 ml and incubation was continued for 10 min at 27°C. NaCl concentration was adjusted to 0.15 M (see Methods), 0.9 ml of substrate was added, and incubation was continued for 60 min at 27°C.

^a SD, SD rat plasma.

^b HP, hepatic perfusate.

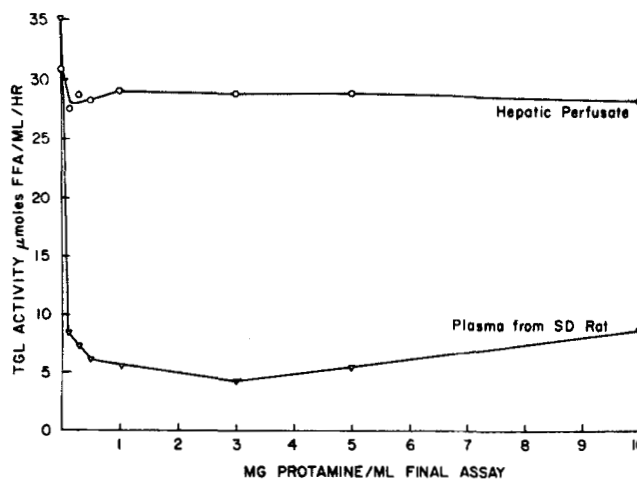


FIG. 5. Effect of protamine concentration on inhibition of TGLA in postheparin hepatic perfusate and plasma from the SD rat. 0.025-ml aliquots of postheparin perfusate or SD rat plasma (one preparation each) were incubated at 27°C for 10 min in 0.1 ml of 0.194 M Tris-HCl buffer, pH 8.6, containing 0.025 ml of heparinized rat plasma, 0.10 M sodium chloride, and the indicated quantities of protamine. This was followed by incubation with 0.9 ml of substrate (final salt concentration 0.15 M) for 60 min at 27°C.

Lipase activities with VLDL as substrate

Lipolysis of VLDL by postheparin hepatic perfusate or SD rat plasma was examined in assays employing either VLDL or triolein as substrate (Table 5). The relative activity against VLDL (VLDL/triolein) of extrahepatic lipase was greater than that of hepatic lipase. Omission of Triton X-100 from the assay in-

TABLE 3. Inhibition of postheparin TGLA with protamine sulfate

Enzyme Source	TGLA		
	-Prot-amine	+Prot-amine	% Inhibition
			μmoles FFA/ml/hr
Hepatic perfusate			
A ^a	30.8	28.8	6.5
B	23.8	21.5	9.7
C	29.0	25.4	12.4
Mean ± SEM			9.5 ± 1.7
SD rat plasma			
A	18.7	1.2	93.6
B	19.5	2.2	88.7
C ^b	36.0	4.2	88.5
D	20.4	1.7	91.7
Mean ± SEM			90.6 ± 1.2

Aliquots of the indicated enzyme preparations (0.025 ml) were incubated for 10 min at 27°C in 0.1 ml of Tris-HCl buffer, I = 0.05, pH 8.6, containing 0.025 ml of heparinized rat plasma and 0.1 M NaCl or 0.1 M NaCl plus 3 mg of protamine. Triolein emulsion (0.9 ml) was added and incubation was continued for 60 min at 27°C.

^a Means of six determinations.

^b Means of two determinations.

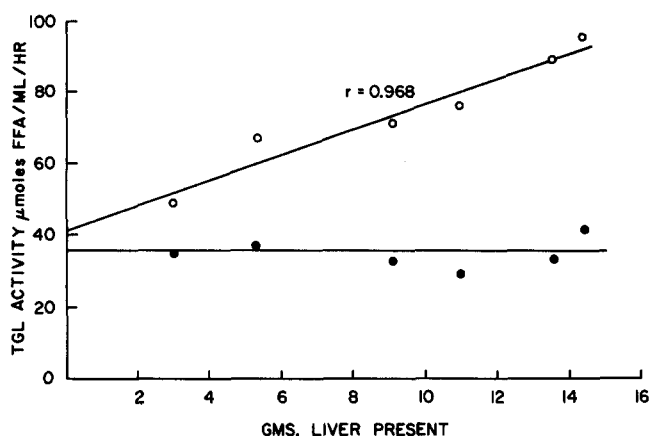


FIG. 6. Estimation of extrahepatic and hepatic TGLA in postheparin plasma from rats with varying degrees of hepatectomy. Aliquots of postheparin plasma (0.01 ml) from rats with varying degrees of hepatectomy (0–80%; see text) were preincubated for 10 min at 27°C with 0.1 ml of 0.194 M Tris-HCl buffer, I = 0.05, pH 8.6, containing 0.1 M NaCl or 0.1 M NaCl plus 3 mg of protamine. After preincubation, 0.9 ml of substrate was added (final salt concentration 0.15 M), and incubation was continued for 60 min at 27°C. The control activity (○) and protamine-inactivated activity (●) are plotted for each hepatectomized rat. Protamine-resistant activity is represented by the difference between the open and closed circles. Slopes of both lines were calculated from simple linear regression.

creased the activity of each enzyme against VLDL, but the relatively greater activity of extrahepatic lipase was still apparent.

DISCUSSION

A previous report from this laboratory suggested that TGLA in postheparin plasma was heterogeneous and included activity released from the liver (13). This suggestion was based on the similar responses of plasma and hepatic TGLA to inhibitors (NaCl, protamine, and pyrophosphate) and the fact that these responses differed from those of TGLA present in extrahepatic tissues.

In the present study we reexamined the effects of NaCl and protamine on TGLA from different sources using an assay system with a much higher triglyceride concentration to avoid substrate limitation of enzyme activity. Because a high triglyceride concentration can result in protection of TGLA from inhibition by salt and protamine, it was necessary to assay activity after prior incubation of the enzyme with the inhibitor. The reduction of enzyme activity by either protamine or NaCl was shown to depend on the time and temperature of prior incubation. This time-dependent effect of protamine or NaCl may be referred to as enzyme inactivation (21). No reversal of this inactivation could be achieved by dilution or prolonged dialysis. Despite the differences

TABLE 4. Protamine inhibition of TGLA in postheparin plasma from SD rat and hepatic perfusate and their mixtures

Enzyme Source ^a	TGLA μmoles FFA/ ml assay/hr
Expt. 1	
HP	0.58
SD	0.73
HP + SD	1.24
HP + SD + protamine	0.54
Protamine-inactivated activity	0.70
Expt. 2	
HP	0.59
SD	0.62
HP + SD	1.21
HP + SD + protamine	0.55
Protamine-inactivated activity	0.66

Assays were carried out as described in Table 2. Total enzyme volume (HP + SD) was 0.05 ml. Experiments 1 and 2 employed plasma from two different rat SD preparations.

^a HP, hepatic perfusate; SD, SD rat plasma.

in the assays used, the effects of inhibitors on plasma, hepatic, and adipose tissue TGLA observed in the present study were consistent with the inference of LaRosa et al. (13) that the liver contributes to postheparin plasma TGLA.

Further experiments were designed to obtain conclusive evidence that hepatic TGLA is present in postheparin plasma and to quantify the hepatic and extrahepatic components of the total plasma TGLA measured at pH 8.6. From experiments with the SD rat preparation, it was established that an infradiaphragmatic source contributed to postheparin plasma TGLA. Like the TGLA in hepatic perfusate, TGLA in plasma from the intact rat was relatively resistant to inactivation by NaCl and protamine. In contrast, the marked inactivation of TGLA in plasma from the SD rat closely resembled that of TGLA in rat adipose tissue. It was further shown that the nearly complete inactivation of SD rat TGLA by protamine was not altered by the addition of hepatic perfusate to SD rat plasma. The marked increase of TGLA observed at higher salt concentrations, first reported by LaRosa et al. (13), is as yet unexplained. This increase occurred at lower NaCl concentrations for hepatic than for extrahepatic TGLA and was associated with resistance of hepatic and plasma TGLA to protamine inactivation.

The SD rat provided a means for characterizing the extrahepatic contribution to TGLA in postheparin plasma; the hepatic TGLA in liver perfusate was used for comparison. Assay conditions were chosen to achieve simultaneously the maximum possible reduction (91%) of extrahepatic TGLA by protamine with minimal de-

TABLE 5. FFA release from triolein and human VLDL incubated with hepatic perfusate or postheparin plasma from the SD rat

Enzyme Source ^a	VLDL Source ^b	Triton X-100	Lipase Activity		VLDL/Triolein
			VLDL	Triolein	
			<i>μmoles FFA/ml/hr</i>		
Hepatic perfusate ₁	Type V (1)	—	12.3	21.6	0.57
Hepatic perfusate ₁	Type III	—	8.6	19.5	0.44
Hepatic perfusate ₂	Type V (2)	—	7.9	16.8	0.47
Mean ± SEM					0.49 ± 0.04
SD rat plasma ₁	Type V (1)	—	16.2	19.4	0.84
SD rat plasma ₁	Type III	—	9.9	15.3	0.65
SD rat plasma ₂	Type V (2)	—	26.4	33.4	0.79
Mean ± SEM					0.76 ± 0.06
Hepatic perfusate ₁	Type V (1)	+	5.9	22.1	0.27
SD rat plasma ₁	Type V (1)	+	8.9	19.0	0.47

0.025 ml of plasma or perfusate was incubated with either triolein or VLDL (8.5 μmoles/ml) in a total volume of 1 ml under the conditions otherwise described in the text. Triton X-100 (0.045% v/v) was present in all assays with triolein, but was omitted from the assays with VLDL where indicated.

^a Subscripts indicate preparations of enzyme from different rats.

^b Numbers in parentheses indicate VLDL prepared from two different type V patients.

pression (10%) of TGLA released from liver. This assay was then used to measure extrahepatic TGLA in postheparin plasma by selective inactivation. The validity of the assay was confirmed using postheparin plasma from partially hepatectomized rats. Protamine-resistant activity was directly proportional to the amount of hepatic tissue present. Protamine-inactivated activity, on the other hand, was independent of the amount of liver removed. The results established that liver is the major, and possibly the sole, source of protamine-resistant TGLA in postheparin plasma and that extrahepatic TGLA can be measured in terms of the protamine-inactivated activity.

The measurement of protamine-inactivated and protamine-resistant TGLA, however, does not provide a precise quantitation of extrahepatic and hepatic enzyme activities. It is impossible to inactivate extrahepatic TGLA completely without some reduction of hepatic TGLA. A correction may be made for this discrepancy.⁴

The measurement of the two components of plasma TGLA by selective inactivation has been used in clinical investigations. Patients with familial type I hyperlipoproteinemia were found to have a gross deficiency of protamine-inactivated TGLA while protamine-resistant TGLA was normal in most cases (22).

⁴ Protamine-resistant TGLA = 0.1(extrahepatic TGLA) + 0.9(hepatic TGLA); protamine-inactivated TGLA = 0.9(extrahepatic TGLA) + 0.1(hepatic TGLA). Thus, hepatic TGLA = 1.125(protamine-resistant TGLA) - 0.125(protamine-inactivated TGLA); and, extrahepatic TGLA = 1.125(protamine-inactivated TGLA) - 0.125(protamine-resistant TGLA). For the activities measured in rat postheparin plasma (Fig. 6), the corrections introduced by these formulas fall within the experimental error of the assay.

As suggested previously (13), the differences that we have found between hepatic and extrahepatic TGLA do not necessarily imply that two different enzyme proteins are involved. It is conceivable that a different conformation, macromolecular aggregate, prosthetic group, or other feature affects the responses of a single enzyme to inhibitors that serve here to distinguish the two lipase activities in postheparin plasma. It has been shown recently by Iverius et al. (21) that the presence of heparin impeded the inactivation of bovine milk lipoprotein lipase by NaCl and protected the enzyme from inhibition by serum. We have observed a similar protective effect of added heparin (1.5-500 U/ml) on salt inactivation of TGLA from SD rat plasma, but there was no effect on the inactivation of TGLA from hepatic perfusate. The addition of heparin did not protect either enzyme activity from inactivation by protamine except when the concentration of heparin was so high that ionic binding of much or all of the protamine in the assay might be expected. Thus, small variations in the heparin content of postheparin plasma may influence the inactivation produced by NaCl but not by protamine. These results, however, do not rule out the possible role of small quantities of enzyme-associated heparin in determining the properties of these enzymes in postheparin plasma.

In a recent report, published after the completion of the present studies, Zieve and Zieve (23) found no reduction in postheparin plasma TGLA in rats after hepatectomy performed as a two-stage procedure. Their conclusion that postheparin plasma lipase originates entirely from extrahepatic tissues is in contradiction to our findings. We believe the failure of Zieve and Zieve (23) to detect hepatic TGLA in postheparin plasma

is most likely a consequence of the assay system they employed. The substrate concentration (1.1 mM triolein) was substantially lower than that which we have found to be necessary to achieve maximal activity. The TGLA they measured in plasma from the intact rat was 10- to 15-fold lower than that we observed after comparable doses of heparin. Another factor that may have produced lower activity of a hepatic TGLA component was the addition of human serum to their assay, which may have resulted in inhibition of hepatic TGLA by serum high density lipoprotein as suggested by the data of LaRosa et al. (13). We have found that this inhibition is substrate dependent; none was observed when the substrate triglyceride concentration was saturating. Finally, Zieve and Zieve (23) performed hepatectomy using a procedure different from ours; thus, their results may not be directly comparable.

The finding that in our assay hepatic TGLA has a relatively high activity in rat postheparin plasma arouses speculation about the physiological role of this enzyme. Liver has been implicated in chylomicron and VLDL triglyceride clearance (24-26). It has been suggested that the quantitative importance of this hepatic function is small and may be limited to uptake of small quantities of triglyceride in lipoprotein "remnants" (27, 28). Furthermore, estimates of the ability of hepatic lipase to catalyze hydrolysis of lipoprotein triglyceride have varied considerably (1, 2, 13, 29-31). In the current experiments it was observed that the relative hydrolytic activity of hepatic lipase against VLDL was approximately two-thirds that of extrahepatic lipase. It was previously reported from this laboratory that VLDL was comparatively a much poorer substrate for hepatic lipase (13). The assay conditions were different from those used here and no direct comparison is possible. The present data, however, raise the possibility that both hepatic and extrahepatic TGLA may be involved in lipoprotein catabolism.

The authors express their appreciation to Mrs. Laura Miller and Mr. Albert Spaeth for their excellent technical assistance.

Manuscript received 5 September 1972; accepted 28 December 1972.

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