

# Lipoprotein lipase-like activity in the liver of mice with Sarcoma 180

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**Abstract** The triglyceride lipase (TGL) activity of liver homogenates of mice with Sarcoma 180 was measured. The liver homogenate of normal or tumor-bearing mice was treated with 0.25% Triton X-100 and centrifuged at 100,000 g for 60 min, and the supernatant was applied to a heparin-Sepharose column. In normal mice, most of the TGL activities in the supernatant was eluted with 0.75 M NaCl from the column. In mice with Sarcoma 180, the TGL gave two peaks on heparin-Sepharose column chromatography, which were eluted with 0.75 M and 1.5 M NaCl, respectively. The activity in the first peak (0.75 M NaCl eluate) decreased; that in the second peak (1.5 M NaCl eluate) increased, and the ratio of the second peak to the first peak increased during tumor development. The livers of normal mice and mice on day 10 after tumor inoculation were perfused with heparin. The highest rate of the TGL release occurred within 1 min of heparin perfusion, and the bulk of heparin-releasable activity appeared within 2 min of perfusion in both normal and tumor-bearing mice. The TGL activity in liver perfusate of tumor-bearing mice, as well as that of liver homogenate, was resolved on a heparin-Sepharose column into two peaks, which were eluted with 0.75 M and 1.5 M NaCl, and most of the activity was eluted with 1.5 M NaCl. The nature of the TGL activity eluted from a heparin-Sepharose column was investigated. In both liver homogenates and liver perfusates, the first peak did not require serum for maximal activity and was relatively resistant to a high concentration of NaCl or protamine sulfate. The second peak required serum for maximal activity and was inhibited by NaCl and protamine sulfate. ■ These results suggest that in the liver of mice with Sarcoma 180, the hepatic TGL activity decreases and a lipoprotein lipase-like activity increases during tumor development.—Masuno, H., T. Tsujita, H. Nakanishi, A. Yoshida, R. Fukunishi, and H. Okuda. Lipoprotein lipase-like activity in the liver of mice with Sarcoma 180. *J. Lipid Res.* 1984. 25: 419–427.

**Supplementary key words** triglyceride lipase • lipoprotein lipase • heparin-Sepharose

Striking depletion of body lipid is frequently observed in tumor-bearing animals and patients with cancer (1–4), but the reason for this depletion is not fully understood. Several possible mechanisms could be involved, including change in the rate of lipid deposition or of lipid mobilization, or both. One observed change is decrease in LPL activity of adipose tissue (4, 5). This change occurs in a tissue-specific manner. Thompson et al. (4) reported

that a decrease in LPL activity of adipose tissue is accompanied by increases in activity in the heart and in the tumor itself. Similarly, a tissue-specific change of LPL activity has been noted in starved rats, in which the activity decreases in adipose tissue and increases in the heart (6), and in lactating rats, in which the activity decreases in adipose tissue but increases in the mammary gland (7). Since there are few reports on the TGL activity in the liver of tumor-bearing animals, we measured the TGL activity in the liver of mice with Sarcoma 180.

Intravenous injection of heparin in the rat and in humans causes release of two TGLs into the plasma, one originating in extrahepatic tissues and designated LPL, and the other originating probably in the liver and named hepatic TGL, which can be also identified in perfusates of the isolated rat liver after the addition of heparin (8). Hepatic TGL has an alkaline pH optimum, is not inhibited by 1 M NaCl, and does not require an apolipoprotein cofactor (8–10). In contrast, LPL has an alkaline pH optimum, is inhibited by 1 M NaCl, and requires an apolipoprotein cofactor for maximal activity (11–15). These enzymes can be easily separated by affinity chromatography on heparin-Sepharose (10, 16).

In the present investigation, we demonstrated that the hepatic TGL activity decreased and the LPL-like activity increased in the livers of mice during tumor development.

## MATERIALS AND METHODS

### Materials

Tri[1-<sup>14</sup>C]oleoylglycerol (30–60 mCi/mmol) and ACS-II were purchased from Amersham, Arlington

Abbreviations: TGL, triglyceride lipase; LPL, lipoprotein lipase; EDTA, trisodium ethylenediaminetetraacetate, trihydrate; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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Heights, IL. Triolein was obtained from Sigma Chemical Co., St. Louis, MO. Bovine albumin (fraction V from bovine plasma) was obtained from Armour Pharmaceutical Co., Phoenix, AZ. Heparin-Sepharose CL-6B was obtained from Pharmacia Fine Chemicals, Sweden. Heparin from porcine intestinal mucosa was obtained from Novo Industri A/S., Copenhagen, Denmark. A kit for radioimmunoassay of plasma immunoreactive insulin was obtained from Dainabot, Tokyo, Japan. Kits for the triglyceride C II-test and cholesterol B-test were purchased from Wako Pure Chemicals, Ltd., Osaka, Japan. Other chemicals were products of Wako (Japan).

### Tumor

The initial inoculum of Sarcoma 180 tumor cells was obtained from Drs. G. Funatsu and N. Yamasaki (Laboratory of Biochemistry, Faculty of Agriculture, Kyushu University, Fukuoka, Japan). Sarcoma 180 tumor cells were maintained by intraperitoneal injection of approximately  $5 \times 10^9$  cells in 0.5 ml of 0.15 M NaCl into male ICR/JCL mice (5 weeks old). The tumors were transferred every 10 days. Over 90% of the mice died within 13–14 days after inoculation of Sarcoma 180.

### Preparation of Sarcoma 180 cell homogenate

Sarcoma 180 cells were harvested 10 days after inoculation and centrifuged at 400 *g* at 4°C for 5 min. The fluid was siphoned off and the cells were resuspended in water for 30 sec, according to the procedure of Katchman, Zipf, and Murphy (17) to hemolyze contaminating erythrocytes. After 30 sec, a large volume of 0.15 M NaCl was added and the cells were centrifuged at 400 *g*. A total of three such washing procedures was used. The cells were then suspended in an equal volume of ice-cold solution of 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA (pH 7.4) and disrupted by sonication at 0°C in an ultrasonic disruptor (model UR-200P, Tomy Seiko Co., Ltd., Tokyo) at position 4 for four 10-sec periods.

### Preparation of liver homogenate

Mice were fed ad libitum and were killed by decapitation between 9:00–9:30 on the day of the experiment. The liver was promptly removed and washed in ice-cold 0.15 M NaCl. Homogenates (25%, w/v) were prepared in ice-cold solution of 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, in a glass-Teflon pestle homogenizer.

### Liver perfusion

Mouse livers were perfused in situ in a nonrecirculating system with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine albumin and 200 mg/dl glucose. Under ether anesthesia the portal vein and the vena cava, above the entrance of the hepatic vein, were cannulated.

After ligation of the inferior vena cava, above the entrance of the right renal vein, and ligation of the splenic vein, the liver was flushed with 20 ml of 0.15 M NaCl and the cannula was connected to the perfusion apparatus. Non-recirculating perfusion was carried out by gravity flow from a reservoir kept at 37°C and placed 30 cm above the cannula situated in the portal vein. The perfusion fluid was aerated with O<sub>2</sub>-CO<sub>2</sub> 95:5 and the flow rate was 10 ml/min. After a preliminary 2-min perfusion, heparin was injected into the reservoir to give a final concentration of 10 or 50 units/ml. The perfusion fluid which passed through the liver was collected in ice-cooled tubes which were changed at intervals of 0–1, 1–2, 2–3, 3–4, and 4–5 min and aliquots (0.2 ml) were taken for determination of enzyme activity.

### Measurement of triglyceride lipase

Substrate emulsions were prepared freshly on the day of use as follows. Tri[1-<sup>14</sup>C]oleoylglycerol (2.5 μCi) was sonicated with 50 mg of triolein in 3.75 ml of neutral gum arabic solution (5%, w/v) in an ice bath. The buffer used in the assay system consisted of 9.1 ml of Tris-HCl buffer (pH 8.4), 3.6 ml of 16% bovine albumin, and 4 ml of heat-inactivated (56°C, 10 min) human serum, as a source of apolipoprotein activator, or 4 ml of deionized water. The assay system contained the following components in a total volume of 0.503 ml: 0.05–0.2 ml of enzyme solution, 0.036 ml of tri[1-<sup>14</sup>C]oleoylglycerol substrate emulsion, and 0.167 ml of the above buffer. In inhibition tests, 0.1 ml of inhibitor (NaCl or protamine sulfate) was added to this assay system. Reactions at 37°C were started by adding the substrate emulsion and stopped after 60 min by adding 6.5 ml of methanol-chloroform-n-heptane 1.41:1.25:1.00 (v/v/v) (18). To this mixture 2.1 ml of 50 mM potassium bicarbonate buffer (pH 10.5) was then quickly added; the tube was shaken vigorously and centrifuged at 1,000 *g* for 10 min. Two-ml aliquots of the upper phase were transferred to scintillation vials and counted in a Packard tri-carb scintillation spectrometer (model 3330) with 10 ml of ACS-II. One unit of the TGL activity was equivalent to the release of 1 μmol of oleic acid per hr.

### Heparin-Sepharose column chromatography

Heparin-Sepharose column chromatography was carried out by the method of Chung and Scanu (19) with slight modification. All buffers used in this experiment contained 30% glycerol (v/v). The sample solution was applied to a heparin-Sepharose column (0.9 × 8.0 cm) equilibrated with 5 mM veronal buffer (pH 7.0) containing 0.4 M NaCl. The column was eluted sequentially with 60 ml of veronal buffer (pH 7.0) containing 0.4 M NaCl, 30 ml of veronal buffer (pH 7.0) containing 0.75 M NaCl, and 30 ml of 1.5 M NaCl-veronal buffer (pH 7.0), and

1.5-ml fractions of eluate were collected. Aliquots (100  $\mu$ l of 0.75 M NaCl eluates and 50  $\mu$ l of 1.5 M NaCl eluates) of fractions were used for assay of the TGL activity. Enzyme was assayed immediately after column fractionation. The ionic strength after the addition of the column fractions to the assay medium was 0.15.

#### Protein determination

Protein was measured by the method of Lowry et al. (20).

#### Statistics

Data were analyzed by Student's *t*-test.

## RESULTS

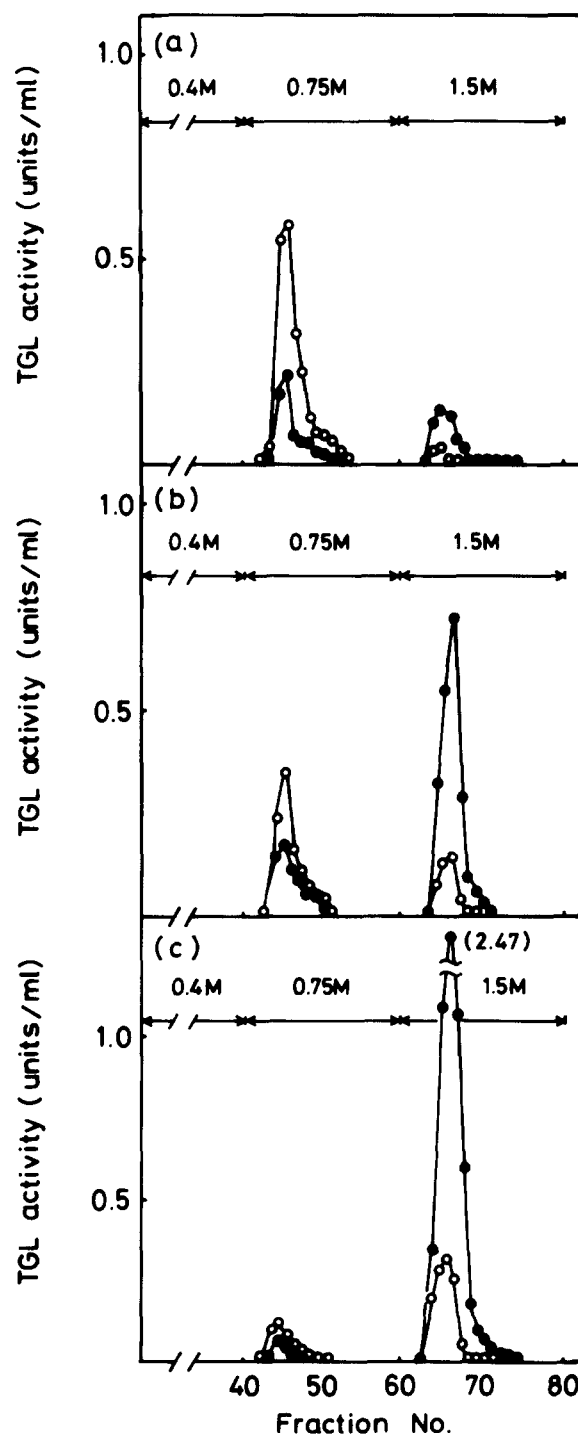
### TGL activity of liver homogenates of mice

On assay of the TGL activity of 25% liver homogenates, the release of oleate was proportional to the volume of homogenate with up to 0.1 ml, and to time for up to 60 min (data not shown). When the homogenate was centrifuged at 100,000 *g* for 60 min and the TGL activity was measured, little activity was recovered in the supernatant. However, after treatment of the homogenate with 0.25% Triton X-100 for 30 min, 60–70% of the activity was recovered in the 100,000 *g* supernatant.

The TGL activity in the supernatant was resolved by chromatography on heparin-Sepharose into two peaks, which were eluted with 0.75 M and 1.5 M NaCl. The effect of tumor growth on the two lipolytic activities resolved by chromatography on heparin-Sepharose was examined. Typical chromatograms are shown in Fig. 1. Eighty to 85% of the applied TGL activity was retained on the column. The second peak (1.5 M NaCl eluate) was minor in a liver homogenate of normal mice (Fig. 1-a) or of mice on day 2 after tumor inoculation, a time when there were few tumor cells in the peritoneal cavity. However, on day 3 after tumor inoculation, the first peak (0.75 M NaCl eluate) decreased and the second peak increased (Fig. 1-b). On day 10, the second peak accounted for most of the activity (Fig. 1-c). The ratio of the second peak to the first peak increased as the tumor grew (Table 1).

We measured the TGL activity in the livers of starved mice without tumors, since food intake in tumor-bearing mice decreased with development of the tumor (data not shown). The first peak decreased but the second peak did not increase. In the liver of 19-day fetuses of mice, 71.6% of the TGL activity was eluted with 1.5 M NaCl (Table 1).

Qualitatively similar results were obtained with acetone powders although the quantitative recovery of the TGL activity was decreased.



**Fig. 1.** Heparin-Sepharose column chromatography of the TGL activity in liver homogenates. A 25% liver homogenate from mice ( $n = 4$ ) was treated with 0.25% Triton X-100 and centrifuged at 100,000 *g* for 60 min. Seven ml of the supernatant was applied to a heparin-Sepharose column. The TGL activity was eluted and assayed as described in Materials and Methods. Activity was measured in the absence (○) and presence (●) of human serum. (a) Liver homogenate of normal mice; protein applied was 284.7 mg and 92.7% of the applied activity was recovered. (b) Liver homogenate of mice on day 3 after tumor inoculation; protein applied was 296.7 mg and 91.3% of the applied activity was recovered. (c) Liver homogenate of mice on day 10 after tumor inoculation; protein applied was 286.3 mg and 89.4% of the applied activity was recovered.

TABLE 1. Effect of tumor growth on the TGL activity

Days after Tumor Inoculation	Total Activity		Ratio <sup>a</sup>
	First Peak	Second Peak	
	<i>units</i>		
Normal (fed)	3.12	0.49	0.2
Normal (48-hr starved)	2.01	0.37	0.2
2	3.84	0.40	0.1
3	1.92	2.82	1.5
4	1.10	5.53	5.0
8	0.40	5.30	13.3
10	0.37	9.04	24.5
13	0.39	22.50	57.7
Fetal liver <sup>b</sup>	3.53	11.24	3.2

At all stages of tumor growth, 314.4 ± 7.3 mg of protein in 7 ml was applied to the column.

$$^a \text{Ratio} = \frac{\text{total activity of second peak}}{\text{total activity of first peak}}$$

<sup>b</sup> The livers were obtained from the fetuses of five litters and the applied protein was 301.7 mg.

### TGL activity of Sarcoma 180

Sarcoma 180 cells were obtained from four mice on day 10 after inoculation and then homogenized. The TGL activity of the homogenates, in the absence of human serum, was 3.23 ± 1.07 munits/mg of protein. The homogenates were combined, treated with 0.25% Triton X-100, and centrifuged at 100,000 g for 60 min. The 100,000 g supernatant was applied to a heparin-Sepharose column; the TGL activity was not retained.

### TGL activity in liver perfusate

The livers of normal mice and of mice on day 10 after inoculation of Sarcoma 180 were perfused with heparin and the TGL activity in the perfusates was measured. In both normal and tumor-bearing mice, the amount of enzyme activity in the perfusates increased with higher doses of heparin (Table 2). The TGL activity measured in the perfusate prior to the addition of heparin was quite low.

TABLE 2. Effect of heparin concentration on the release of the TGL activity during liver perfusion

Heparin Concentration in Perfusate	Heparin-releasable TGL Activity	
	Normal Mice	Tumor-bearing Mice
<i>units/ml</i>	<i>units</i>	
10	10.0 ± 1.5 (5)	5.9 ± 0.7 (3)
50	16.3 ± 2.3 (3)	7.8 ± 1.2 (3)

A nonrecirculating perfusion was carried out for 5 min after addition of heparin. Total TGL activity in the perfusate pooled over 5 min was measured. The TGL activities in perfusates of normal mouse livers were measured without human serum. The activities in perfusates of the livers of mice on day 10 after tumor inoculation were measured in the presence of human serum. Values are means ± SE. Figures in parentheses represent the number of experiments.

Addition of heparin to the perfusion fluid resulted in a marked and rapid release of the TGL activity from the liver which was maximal at the 0–1 min interval (Fig. 2). Subsequently, the rate decreased markedly and approached the pre-heparin values during the 4–5 min interval. Human serum had no effect on heparin-releasable TGL activity in liver perfusates of normal mice (Fig. 2-a). The TGL activity in liver perfusates of tumor-bearing mice was strongly activated by human serum (Fig. 2-b). These results suggest that liver perfusates of tumor-bearing mice contained a significant portion of serum-dependent lipolytic activity.

The perfusates were applied to heparin-Sepharose col-

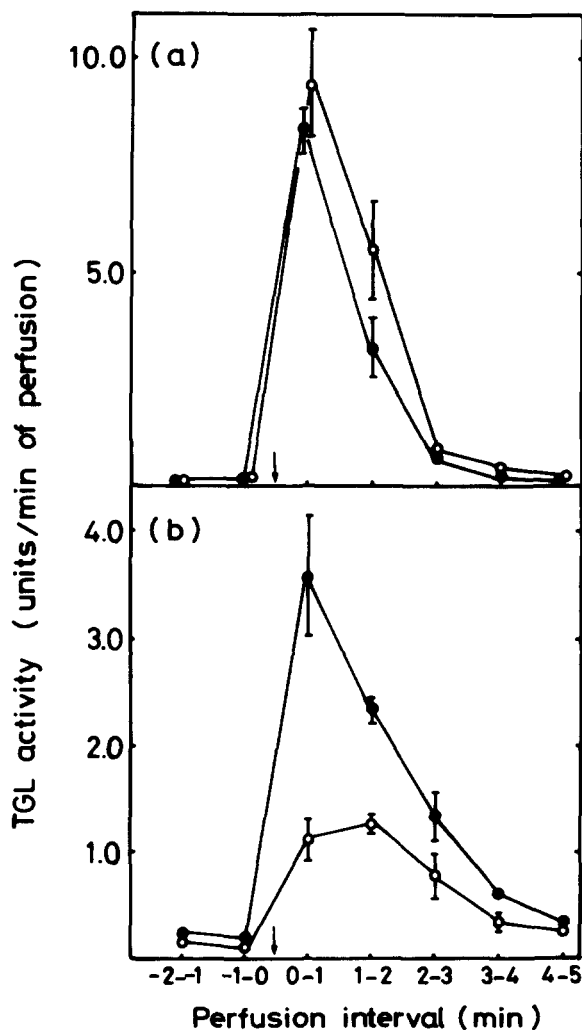


Fig. 2. Heparin-releasable TGL activity in liver perfusates. The livers of normal mice (a) and mice on day 10 after tumor inoculation (b) were perfused in situ in a nonrecirculating system with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine albumin and 200 mg/dl glucose. The arrow shows the time of addition of heparin (50 units/ml) into the perfusion fluid. Values were means ± SE (n = three mice) of the TGL activity released per min determined on samples collected at the stated time intervals. Activity was measured in the absence (○) and presence (●) of human serum.



umns and typical chromatograms are shown in Fig. 3. Seventy to 80% of the applied activity was retained on the columns and the activity was resolved into separate peaks. The first and second peaks were eluted with 0.75 M and 1.5 M NaCl, respectively. In normal mice, most of the activity in the perfusate was eluted with 0.75 M NaCl (Fig. 3-a). In contrast, in tumor-bearing mice the second peak accounted for most of the activity (Fig. 3-b).

#### TGL activity in the liver after heparin perfusion

The TGL activity of homogenates of liver before and after the perfusion of 50 units/ml heparin for 5 min was resolved by chromatography on heparin-Sepharose. In normal mice, the first peak after perfusion decreased to less than 20% of that before perfusion (TGL activity before perfusion, 3.12 units; TGL activity after perfusion, 0.50 units). In mice on day 10 after tumor inoculation, after perfusion the first peak was not detectable and the second peak was reduced to half of that before perfusion (TGL activity in the second peak: before perfusion, 9.04 units; after perfusion, 4.92 units).

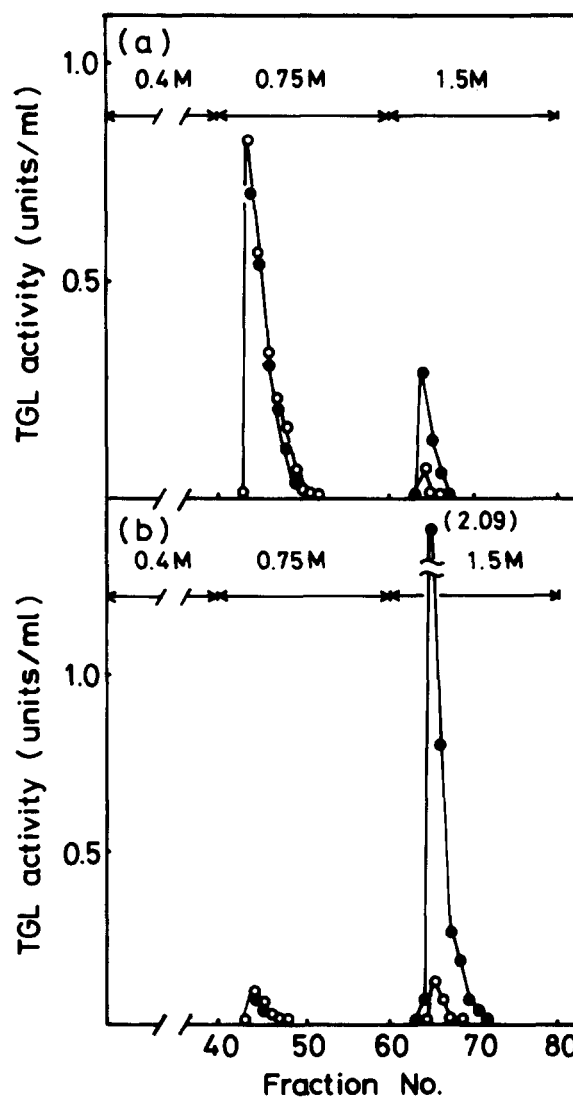
#### Characterization of TGL activity

When liver homogenates were used as a source of the TGL activity, the first peak was strongly inhibited by human serum (Fig. 1). When liver perfusates were used as a source of activity, serum slightly decreased the first peak (Fig. 3). In both cases, the second peak was strongly activated by serum (Figs. 1 and 3) and increased concomitantly with the volume of serum in the assay system (data not shown).

The differences in sensitivities to NaCl and protamine sulfate of the first peak obtained from normal mice and of the second peak obtained from mice on day 10 after tumor inoculation were studied (Table 3). The first peak was relatively resistant to a high concentration of NaCl or protamine sulfate, whereas the second peak was completely inhibited by the presence of 0.9 M NaCl or 3 mg/ml protamine sulfate.

#### Histological study of the livers

The histological features of the livers of normal ( $n = 4$  mice) and tumor-bearing mice ( $n = 4$  mice) were examined. Samples from the livers, 5 mm or less thick, were fixed in 10% formalin, embedded in paraffin, sectioned at 5  $\mu\text{m}$ , and routinely stained with hematoxylin and eosin. Hepatic tissues of tumor-bearing mice were histologically similar to those of normal mice (Figs. 4-a and 4-b). Neither metastasis nor infiltration was observed in the livers of tumor-bearing mice, although there were a few Sarcoma cells in the vascular spaces (arrow). The livers of tumor-bearing mice were flushed with 0.15 M NaCl (20 ml), fixed in 10% formalin, and processed for



**Fig. 3.** Heparin-Sepharose column chromatography of heparin-releasable TGL activity in liver perfusates. A nonrecirculating perfusion was carried out for 3 min after addition of heparin, and 25 ml of the perfusate was applied to a heparin-Sepharose column. The TGL activity was eluted and assayed as described in Materials and Methods. Activity was measured in the absence (O) and presence (●) of human serum. (a) Liver perfusate of a normal mouse; 89.7% of the applied activity was recovered. (b) Liver perfusate of a mouse on day 10 after tumor inoculation; 92.4% of the applied activity was recovered.

the histological examination. Almost all Sarcoma cells were eliminated from the vascular spaces (Fig. 4-c).

#### Changes in plasma levels of lipids, glucose, and insulin of tumor-bearing mice

Table 4 shows the plasma concentrations of triglyceride, cholesterol, glucose, and insulin during growth of Sarcoma 180 in mice. The plasma triglyceride concentration reached a maximum between days 6 and 8 after tumor inoculation and then decreased. The plasma cholesterol concentration increased on day 8. The triglyceride

TABLE 3. Effects of NaCl and protamine sulfate on the TGL activity

	TGL Activity			
	Liver Homogenate		Liver Perfusate	
	First Peak	Second Peak	First Peak	Second Peak
	<i>units/mg protein</i>			
No addition (control)	3.95 (100.0) <sup>a</sup>	7.87 (100.0)	4.07 (100.0)	7.06 (100.0)
NaCl				
0.2 M	3.59 (91.0)	4.12 (52.3)	3.61 (88.7)	4.28 (68.3)
0.9 M	3.10 (78.5)	0.22 (2.8)	3.26 (80.1)	0.15 (2.1)
Protamine sulfate				
0.25 mg/ml	3.64 (92.2)	1.12 (14.2)	3.56 (86.7)	1.48 (21.0)
3.0 mg/ml	3.53 (89.4)	0.16 (2.0)	3.61 (88.7)	0.32 (4.5)

<sup>a</sup> Percent of the control.

concentration in the liver homogenate increased 4 days after tumor inoculation (liver of normal mice,  $91.1 \pm 3.6$  mg/g liver; liver of tumor-bearing mice,  $120.5 \pm 8.8$  mg/g liver;  $P < 0.01$ ). The plasma glucose concentration decreased as the tumor developed. On day 8 after tumor inoculation the plasma insulin level was decreased significantly; it was also decreased between days 10 and 12, but the decrease was not significant because the changes were small and the values were very variable.

## DISCUSSION

The present study demonstrates the presence of two distinct TGLs in liver homogenates and liver perfusates of mice with Sarcoma 180. One resembles hepatic TGL and the other exhibits properties similar to those of LPL. (Table 3). The hepatic TGL activity accounts for most of the activity and the LPL-like activity was minor in the livers of normal mice. In tumor-bearing mice, the hepatic TGL activity decreased and the LPL-like activity increased with development of the tumor (Table 1).

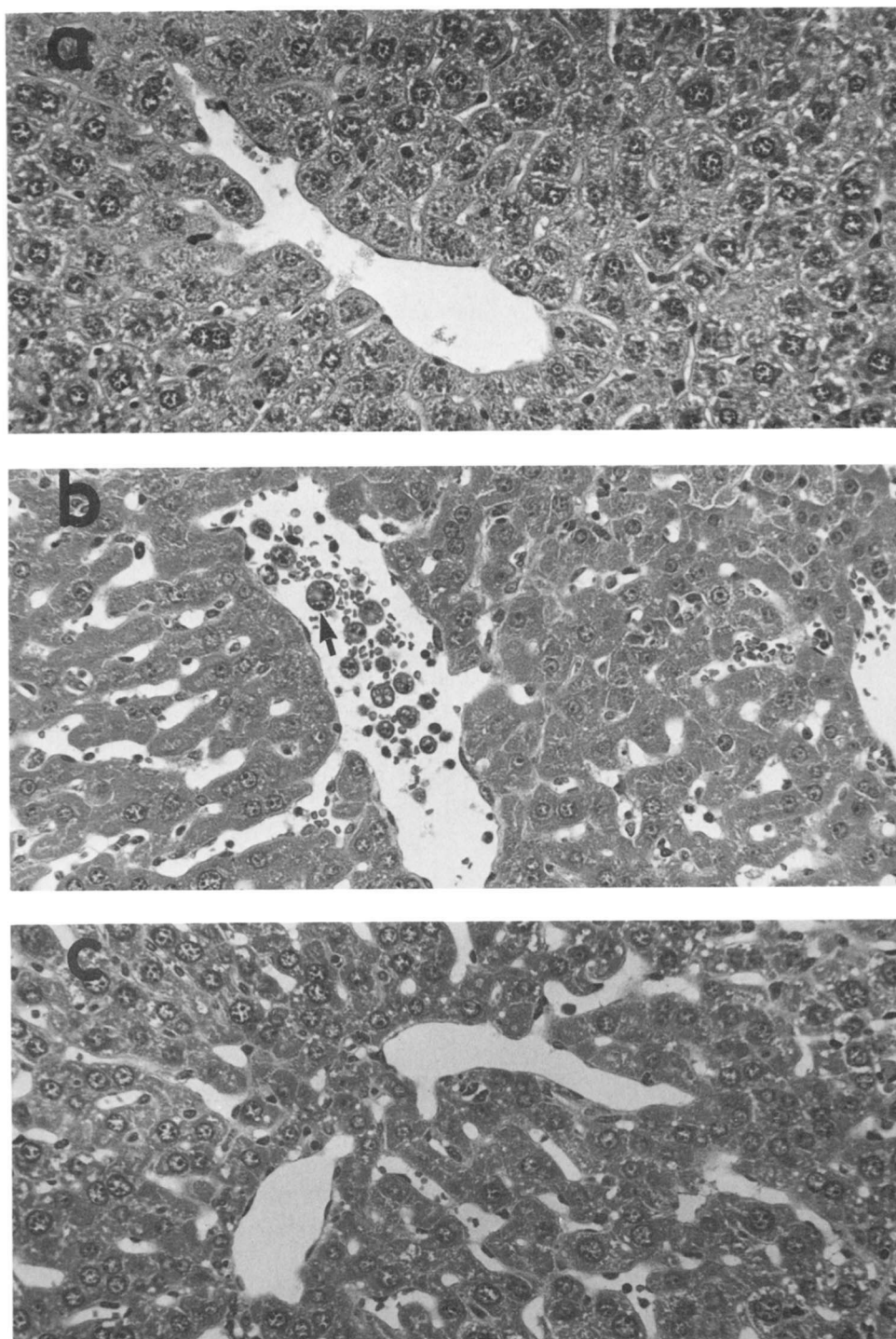
The mechanisms of decrease in the hepatic TGL activity and increase in the LPL-like activity in the livers of tumor-bearing mice remain to be elucidated.

Jansen et al. (21) reported that parenchymal cells are the site of hepatic lipase synthesis. In this study, we showed that Sarcoma 180 cells did not infiltrate into the liver, and hepatic tissues of normal and tumor-bearing mice displayed similar histological features (Fig. 4). Neither the hepatic TGL activity nor the LPL-like activity can be found in Sarcoma 180 cells. These results suggest that decrease in the hepatic TGL activity was not due to the damage of parenchymal cells by infiltration of Sarcoma 180 cells, and increase in the LPL-like activity was not due to activity produced by Sarcoma 180 cells.

In both normal and tumor-bearing mice, the highest

rate of the TGL release from the liver occurs within 1 min of heparin perfusion and the bulk of releasable activity appeared within the first 2 min of perfusion (Fig. 2). The heparin-releasable hepatic TGL amounted to about 85% of that present in the livers of normal mice. In mice on day 10 after tumor inoculation, about 55% of the LPL-like activity present in the livers was released by heparin into the perfusates. These results suggest that these enzymes are present in the liver in at least two compartments, one intracellular and the other situated at a site from which it can be easily released by heparin. Assmann et al. (22) reported that in rat liver the compartment containing the heparin-releasable activity is localized to the plasma membrane fraction. Ikeda et al. (23) reported that about 90% of the triacylglycerol lipase activity of a rat liver microsomal fraction was released by heparin treatment. The liver, however, is not a homogenous tissue; it consists of parenchymal and nonparenchymal cells (mainly Kupffer and endothelial cells). Jansen, Van Berkel, and Hülsmann (24) reported that the heparin-releasable lipase is located mainly at the surface of nonparenchymal cells and the lipase bound in vitro to nonparenchymal cells is largely releasable by heparin. Kuusi et al. (25) reported that the heparin-releasable lipase in the rat liver is located exclusively on the liver endothelial cells and not on parenchymal or Kupffer cells. In these respects, the liver may be analogous to adipose tissue, where LPL is synthesized in one cell type (the fat cells) and then transported to the functional site at the other cell type (the endothelial cells).

Many workers have reported that the hepatic TGL activity in rats decreases during starvation and diabetes (26–28). The suppressed activity of hepatic TGL in diabetic rats is restored by insulin treatment, suggesting that the activity is under hormonal regulation by insulin. In the present work, we also confirmed that the hepatic TGL activity was reduced during starvation (Table 4).



**Fig. 4.** Histological features of the livers. (a) The liver of a normal mouse. (b) The liver of a mouse on day 10 after tumor inoculation without a flush of 0.15 M NaCl. The arrow shows Sarcoma cells. (c) The liver of a mouse on day 10 after tumor inoculation with a flush of 0.15 M NaCl (20 ml).  $\times 250$ .

In tumor-bearing mice, however, the decrease of the hepatic TGL activity occurred even though the plasma insulin level was normal (Tables 1 and 4). These results indicate that in tumor-bearing mice, but not in normal

mice, the changes in the hepatic TGL activity do not necessarily coincide with those of the plasma insulin level.

LPL catalyzes hydrolysis of plasma VLDL- and chylomicron-triglyceride in a variety of tissues, in which the



TABLE 4. Plasma concentrations of lipids, glucose, and insulin<sup>a</sup>

	Days after Tumor Inoculation						
	Normal	2	4	6	8	10	12
Triglyceride (mg/dl)	232.3 ± 29.1 <sup>b</sup>	276.5 ± 40.5	450.0 ± 39.7*	702.1 ± 82.2*	865.7 ± 157.0*	338.7 ± 83.3	296.2 ± 37.6
Cholesterol (mg/dl)	184.5 ± 5.3	198.3 ± 9.2	166.3 ± 12.8	211.0 ± 3.0	246.3 ± 26.5*	172.3 ± 17.1	174.8 ± 10.7
Glucose (mg/dl)	176.0 ± 5.3	165.4 ± 2.5	146.3 ± 22.7	155.0 ± 9.4	109.6 ± 6.6*	82.0 ± 16.8*	94.0 ± 9.6*
Insulin (μunits/ml)	7.1 ± 1.2	13.5 ± 5.5	6.2 ± 1.2	6.1 ± 2.1	3.0 ± 1.1*	4.6 ± 1.5	5.0 ± 2.6

<sup>a</sup> Mice, fed ad libitum, were killed by decapitation and bled between 9:00–9:30.

<sup>b</sup> Mean ± SE (n = seven mice).

\* P < 0.01 (compared with normal mice, Student's *t*-test).

physiological site of action of the enzyme is believed to be the endothelial surface of the capillary (29). However, the metabolic function of hepatic TGL in lipoprotein metabolism is not well understood. Following the initial hydrolysis of VLDL- and chylomicron-triglyceride by LPL, further modification of the particles has been proposed to result from the action of the TGL originating in the liver. Recently, many workers showed that hepatic TGL may play a role in HDL metabolism in the rat (30, 31) and in humans (32). Goldberg et al. (33) reported that hepatic TGL appears to function in a parallel role with LPL in the conversion of VLDL and IDL to LDL. The functional roles of the hepatic TGL and LPL-like activities in the livers of tumor-bearing mice are still unknown. Both enzymes may act in concert in the catabolism of remnants of triglyceride-rich lipoprotein. ■

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