Characterization, Subcellular Localization, and Partial Purification of a Heparin-released Triglyceride Lipase from Rat Liver*

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SUMMARY

Post-heparin plasma in the rat contains triglyceride lipase activity of both hepatic and extrahepatic origin. The lipase released into rat hepatic perfusate by heparin has been characterized in an assay containing Triton X-100, albumin, and [14C]triolein. Fatty acid release was linear for 120 min over a wide range of enzyme concentrations at 27° and 37°. The pH optimum was 9.5. Enzymatic activity was >80% inhibited by prior incubation with 0.5 M NaCl or 750 pg of protamine per ml. The apparent \( K_m \) was 1.28 mM. The enzyme was localized in rat liver cell fractions. Cell fractions were checked for purity by marker enzymes and electron microscopy. Activity inhibited by NaCl or protamine was found in plasma membranes, microsomes, and cytosol, having pH optima of 9.5, 9.0, and 8.0, respectively. A different triglyceride lipase not affected by NaCl or protamine and having a pH optimum at 4.4 was found in lysosomes. The plasma membrane-bound lipase had a specific activity 10 times that in liver homogenate. Addition of heparin to the plasma membrane fractions in a concentration as low as 1 unit per ml effected the release of the enzyme into the medium. The plasma membrane-released lipase had the same apparent \( K_m \). A 360-fold enhancement of plasma membrane lipase activity over that in whole liver homogenate was achieved by heparin affinity chromatography.

EXPERIMENTAL PROCEDURE

Materials

Glyceryl tri-[1-14C]oleate and [2-3H]glyceryl trioleate (Amersham-Searle) were >98% pure as judged by thin layer chromatography (Silica Gel G, solvent system: petroleum ether-ether-acetic acid, 90:10:1); other chemicals were obtained from the following sources: unlabeled triolein (Applied Science); fatty acid-free albumin (Pentex); Triton X-100 (Packard); Triton WR 1339 (Serva, Heidelberg); glucose 6-phosphate, 5'-AMP (Boehringer); cytochrome c from horse heart, type III (Sigma); \( p \)-nitrophenylphosphate (Merck); heparin (sodium) 20,000 U.S.P. units per ml (Upjohn Company); protamine sulfate (saline) (Calbiochem); bovine serum albumin (fraction V, Armour); Bio-Gel P-200 (Bio-Rad).

The pH optima were determined with the following buffers: (ionic strength, \( I = 0.1 \)): sodium citrate buffer, pH 3.0, pH 3.4; sodium acetate buffer, pH 3.8, pH 4.4, pH 5.0, pH 5.6; sodium phosphate buffer, pH 6.6, pH 7.4, pH 8.0; glycine-NaOH buffer, pH 8.6, pH 9.0, pH 9.4, pH 9.5, pH 9.6, pH 10.0, pH 10.4. All chemicals, including buffers, were “analytical” or “certified” grade.

Methods

Rat Liver Perfusion—Isolated livers from fed 400-g male N.I.H. Osborn-Mendel rats were perfused as described previously (22). The perfusate consisted of a 1:1 mixture of defibrinated rat blood and Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin and 0.1% glucose and adjusted to pH 7.4 with Na2CO3. After initiation of perfusion, 2500 units of heparin were added (50 units per ml) and the recycled post-heparin perfusate, in a total volume of about 50 ml, was collected 15 min later. It was initially determined that triglyceride lipase activity was not present in the perfusate.
before heparin was introduced and that heparin released lipolytic activity within 2 min, the maximum being reached by 10 min (23). Aliquots were stored at $-20^\circ$ and were stable for at least 6 months.

**Preparation of Subcellular Fractions**—Livers were homogenized with a Dounce homogenizer and fractionated into nuclear, cytoplasmic, mitochondrial, light mitochondrial, microsomal, and supernatant fractions according to the method of deDuve et al. (24). Lysosomes were prepared according to the procedure of Wattiaux et al. (25). Triton WR 1339 in 0.9% NaCl solution, 1.7 mg per g of body weight, was injected into the tail vein of adult rats weighing about 150 g. After 90 hours the animals were killed, and the livers were quickly removed and minced with scissors. The tissue was homogenized in 8 volumes of 0.25 M sucrose, and the “mitochondrial fraction” was separated by centrifugation over a continuous sucrose gradient (0.8 to 1.6 M) into lysosomes and mitochondria. Plasma membranes were obtained from crude nuclear fractions by the method of Neville (26) as modified by Ray (27). The membranes were collected from the discontinuous sucrose gradients at the 37%/41% (w/w) interface, washed two times with 1 mM NaHCO$_3$, 0.5 mM Ca$^{2+}$ solution and the final pellet taken up in the same buffer solution (usually 5 mg of protein per ml). The yield was 0.5 to 0.75 mg of protein per g of fresh rat liver.

**Analytical Procedures**—In all preparations, the following determinations were performed on each cell fraction: 5'-nucleotidase (28), glucose 6-phosphatase (29), acid phosphatase (30), cytochrome c oxidase (31, 32), protein (33). Inorganic phosphate was measured by the method of Chen (34). Enzyme activities were expressed as micromole of substrate utilized per mg of protein per min.

**Assay of Triglyceride Lipase Activity**—Lipase activity was determined by the method of Krauss et al. (35). Components of the assay and their final concentrations were: glycercyl tri-[1-$^3$H]oleate, 10.5 µM; unlabeled triolein, 8.46 µM; fatty acid-free albumin, 15 mg per ml; and Triton X-100, 4.5 x 10$^{-4}$ ml per ml. In the usual assay these constituents were sonicated in glycine-NaOH buffer, pH 9.5, I = 0.1, and Triton X-100 to the incubation medium was necessary for maximal rates of triglyceride hydrolysis by hepatic lipase. The mixtures were then separated by centrifugation (50,000 x g for 15 min at 4°C) into a membrane pellet and clear supernatant. The membranes were resuspended in buffer to the original concentration, and they and the supernatant fractions were then assayed for lipase activity.

**Electron Microscopy**—Pellets of cell organelles were fixed with cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. These preparations were washed with several changes of buffer, post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, dehydrated, and embedded in Maraglas (37). Sections were cut with an LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined with an RCA EMU-3G electron microscope.

**Heparin Affinity Column**—Affinity chromatography has previously been used by Olivecrona et al. (38) to study the binding of milk lipoprotein lipase to heparin. We prepared a column according to the method of Inman and Dintzis (39), linking heparin covalently to Bio-Gel P-200. Plasma membranes were incubated for 1 hour at 27°C in glycine buffer of I = 0.1 and at pH 9.5. The soluble proteins were then separated from membranes by centrifugation (50,000 x g for 15 min) and applied to the column previously equilibrated with the same buffer system. The column was washed with this buffer until the eluate was free of protein and then eluted in 5-ml fractions by application of a continuous NaCl gradient (40 ml of glycine-NaOH buffer, pH 9.5, I = 0.1, the NaCl concentrations increasing from 0 M to 5 M). The fractions containing triglyceride lipase activity were dialyzed against 5 mM NH$_4$CO$_3$ overnight and lyophilized. Column fractions were monitored by sodium dodecyl sulfate gel electrophoresis with a sulfate-borate discontinuous buffer system (running pH 9.5) (40).

**RESULTS**

**Characterization of Hepatic Cell Fractions**—The purity of the cell fractions was monitored by both enzymatic measurements and electron microscopy. The plasma membrane and endoplasmic reticulum fractions appeared to be about 10% cross-contaminated according to their content of glucose 6-phosphatase and 5'-nucleotidase, respectively (Table I). Lysosomes prepared by continuous gradient were free of mitochondria. The mitochondrial fraction contained acid phosphatase and cytochrome c oxidase activities suggesting <2% contamination with lysosomes. The specific activity of the marker enzyme, 5'-nucleotidase, in the plasma membrane preparations was enriched 16.3-fold over that in the whole homogenate. In electron micrographs the plasma membranes were vesicular and contained junctional complexes; only an occasional patch of rough endoplasmic reticulum was seen.

**Optimization of Lipase Assay Conditions**—The addition of albumin and Triton X-100 to the incubation medium was necessary for maximal rates of triglyceride hydrolysis by hepatic lipase (Table I). The hydrolysis of triolein by liver perfusate and liver cell fractions over a range of protein concentrations of 10 µg to 1000 µg per ml proceeded linearly for 120 min at both 27°C and 37°C. The rate of free fatty acid release by the post-heparin liver perfusate was independent of heparin.
Determination of marker enzymes in different cell fractions

Specific enzyme activities are given in micromoles of product formed per mg of protein per 60 min. Incubations were done as described under "Experimental Procedure." Each value represents an average of four determinations. Standard deviation was less than 10%.

<table>
<thead>
<tr>
<th>Lysosomes</th>
<th>Plasma membranes</th>
<th>Microsomes</th>
<th>Mitochondria</th>
<th>Homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>58</td>
<td>4.7</td>
<td>3.8</td>
<td>0.8</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>7.4</td>
<td>30.4</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>0.52</td>
<td>1.7</td>
<td>16.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>0</td>
<td>0.04</td>
<td>0.06</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Effect of different concentrations of Triton X-100 and albumin in emulsification mixture on free fatty acid release

Aliquots of post-heparin liver perfusate were incubated for 60 min at 27° in glycine buffer I = 0.1, pH 9.5. Substrate medium was sonified in the presence of different amounts of Triton X-100 or albumin. Values are expressed in percentage of maximal hydrolytic activity as determined by free fatty acid release.

<table>
<thead>
<tr>
<th>Triton X-100 concentrations</th>
<th>Hydrolytic activity</th>
<th>Albumin concentrations</th>
<th>Hydrolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>µl/ml sonification mixture</td>
<td>% maximal hydrolytic activity</td>
<td>mg/ml sonification mixture</td>
<td>% maximal hydrolytic activity</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>0.15</td>
<td>87</td>
<td>3</td>
<td>73</td>
</tr>
<tr>
<td>0.30</td>
<td>96</td>
<td>7.5</td>
<td>87</td>
</tr>
<tr>
<td>0.45</td>
<td>100</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>0.75</td>
<td>83</td>
<td>22.5</td>
<td>97</td>
</tr>
<tr>
<td>1.5</td>
<td>50</td>
<td>30</td>
<td>92</td>
</tr>
<tr>
<td>3.0</td>
<td>15</td>
<td>60</td>
<td>71</td>
</tr>
</tbody>
</table>

concentration over a range of 0.5 to 200 units per ml of incubation mixture.

The stoichiometry of [14C]fatty acid and [3H]glycerol release was investigated using glyceryl-[1-14C]trioleate and [2-14C]glyceryl trioleate as substrates. The 14C:3H ratios in di- and mono-glycerides and the radioactivity individually present as [2-3H]glycerol and [14C]fatty acids were determined. When the post-heparin perfusate was used as enzyme source and after incubation at 27° for 60 min, 5% of the triglyceride was hydrolyzed to glycerol and free fatty acids; approximately 0.25% was sonified in the presence of different amounts of Triton X-100 or albumin. Values are expressed in percentage of maximal hydrolytic activity as determined by free fatty acid release. TGL, triglyceride lipase.

TABLE III

Specific triglyceride lipase activity of different rat liver cell fractions at pH 9.5

Each incubation contained in a total volume of 1 ml: 0.9 ml of substrate (see "Methods") in glycine-NaOH buffer, pH 9.5 (I = 0.1), 0.1 ml of enzyme source (100 μg of protein). Heparin-released plasma membrane lipolytic activity was determined in the presence of 25 µl of pre-heparin perfusate. Incubations were for 2 hours at 37°.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Free fatty acid (nmol/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>183</td>
</tr>
<tr>
<td>100,000 X g supernatant</td>
<td>223</td>
</tr>
<tr>
<td>Microsomes</td>
<td>458</td>
</tr>
<tr>
<td>Plasma membranes</td>
<td>1790</td>
</tr>
<tr>
<td>Heparin-released plasma membrane lipase</td>
<td>7700</td>
</tr>
</tbody>
</table>

are shown in Table III. When corrected for contamination based on measurements of the marker enzymes (Table I), the specific activity of the lipase was about 8-fold greater in the plasma membranes than in cytosol and microsomes. Because the bulk of the cell protein is present in cytosol, the majority of lipase activity in the alkaline pH range is probably located in this cell fraction. It is not possible, however, to measure the amount of possible contamination of the cytosol with lipase of much higher specific activity released from the plasma membranes during cell fractionation.

We were unable to demonstrate significant lipase activity in mitochondria. Lipase activity at acid pH paralleled the distribution of acid phosphatase throughout the fractionation procedure and appeared to be exclusively of lysosomal origin.

Effects of Heparin on Plasma Membrane Fractions—Since
lipase activity is normally released into the post-heparin perfusate at pH 7.4, investigation of the release of lipase activity by heparin in vitro was carried out at this pH. Plasma membranes obtained from the discontinuous sucrose gradient were washed twice with a solution containing 1 mM NaHCO₃ and 0.5 mM Ca²⁺, and the final pellet was resuspended in the same medium at a protein concentration of 5 mg per ml. As shown in Fig. 2, the addition of small amounts of heparin to the plasma membrane suspension caused the release of significant amounts of hydrolytic activity into the incubation medium. Prior incubation with heparin slightly increased the total lipase activity in plasma membranes (resuspended to the original concentration).

Heparin-released triglyceride lipase from plasma membranes

Plasma membranes suspended in 1 mM NaHCO₃, 0.9 mM Ca²⁺ (protein concentration 5 mg per ml) were divided into five aliquots. Aliquot 1 was preincubated for 15 min at 27°C without further treatment. Aliquot 2 was preincubated for 15 min at 27°C in the presence of 20 units of heparin per ml. Aliquot 3 was treated in the same way as Aliquot 1 except that after the end of preincubation plasma membranes and supernatant were separated by centrifugation (15 min; 4°C; 50000 X g); the plasma membrane pellet was resuspended with incubation medium to the original concentration. Aliquot 4 was treated in the same way as Aliquot 2 except that after the end of preincubation 20 units of heparin were added to the supernatant, precipitation was continued at 27°C for further 5 min. One hundred microliters of each aliquot were taken at the end of preincubation respective centrifugation and incubation continued for 120 min at 37°C by adding 0.9 ml of substrate. Each number is the mean of two experiments (four determinations).

<table>
<thead>
<tr>
<th>Aliquot</th>
<th>Free fatty acid (nmol/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>918</td>
</tr>
<tr>
<td>2</td>
<td>1005</td>
</tr>
<tr>
<td>3</td>
<td>Supernatant</td>
</tr>
<tr>
<td>4</td>
<td>Plasma membranes</td>
</tr>
<tr>
<td>5</td>
<td>Supernatant</td>
</tr>
</tbody>
</table>

The activity of triglyceride lipase in the post-heparin perfusate and liver cell fractions after preincubation with NaCl, over a wide range of concentrations, is shown in Table V. Maximum activity was usually obtained in the absence of added NaCl, but prior incubation of the liver perfusate and microsomes with very high NaCl concentrations (2.5 x and 3.75 x) augmented enzymatic activity above the base-line. The lipase activities in microsomes, cytosol, liver perfusate, and that released from the microsomes were inhibited from 80 to 90% by prior incubation in NaCl at concentrations of 0.31 or 0.63 M. The acid lysosomal lipase activity was inhibited less than 10% at the same salt concentrations. At NaCl concentrations greater than 0.63 M, lipases from different sources other than lysosomes showed a progressive increase in activity. The increase in lipase activity at higher salt concentrations has been previously observed (1) and remains unexplained.

The effects of increasing protamine sulfate concentration on the several lipase activities are illustrated in Table VI. There was no appreciable inhibition of lysosomal triglyceride lipase, but small concentrations of protamine reduced the activity of the other enzyme preparations by 60 to 85%. The degree of inactivation was dependent upon the duration of prior incubation with the inhibitor (Table VII). The concentration of

![Fig. 2. Effect of heparin on the release of triglyceride lipase from plasma membranes. Plasma membranes were incubated in a 1 mM NaHCO₃, 0.5 mM Ca²⁺ solution for 15 min at 27°C in the presence of different heparin concentrations. Incubation volume was 1 ml. Subsequently plasma membranes were sedimented for 15 min (4°C) at 50000 x g and 100 µl aliquots of the supernatant and the plasma membranes (resuspended to the original concentration) determined for lipolytic activity (incubation for 120 min at 37°C). ▲—▲, lipolytic activity in the supernatant; O—O, represents residual lipolytic activity in plasma membranes. FFA, free fatty acid.](http://www.jbc.org/index2.php)
Inhibition of post-heparin liver perfusate triglyceride lipase and different cell fractions with sodium chloride

Aliquots of the different enzyme sources were preincubated for 60 min at 27°C in a total volume of 0.2 ml with glycine buffer (I = 0.1), pH 9.5, containing the indicated concentrations of sodium chloride. Following preincubation, a 0.05-ml aliquot was taken and adjusted to a concentration of 2.0 M NaCl in a volume of 0.1 ml. Then 0.9 ml of substrate mixture containing no salt was added so that the final salt concentration was 0.20 M, and incubation was continued for 60 min at 37°C. Inhibition of lysosomal triglyceride lipase was determined at pH 4.4 (acetate buffer, I = 0.1).

<table>
<thead>
<tr>
<th>Sodium chloride</th>
<th>Hydrolytic activity of different fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-heparin liver perfusate</td>
</tr>
<tr>
<td>% maximal activity</td>
<td>% maximal activity</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.078</td>
<td>91</td>
</tr>
<tr>
<td>0.356</td>
<td>75</td>
</tr>
<tr>
<td>0.312</td>
<td>22</td>
</tr>
<tr>
<td>0.025</td>
<td>18</td>
</tr>
<tr>
<td>0.035</td>
<td>22</td>
</tr>
<tr>
<td>2.5</td>
<td>120</td>
</tr>
<tr>
<td>3.75</td>
<td>120</td>
</tr>
</tbody>
</table>

Table VI

Inhibition of lipolytic activity of post-heparin liver perfusate and different cell fractions with protamine

Aliquots of the different enzyme sources were incubated for 60 min at 27°C in a total volume of 0.2 ml with glycine buffer (I = 0.1), pH 9.5, containing the indicated concentrations of protamine. Following preincubation, a 0.05-ml aliquot was taken and adjusted to 0.1 ml with glycine buffer and incubation continued for 60 min at 37°C by adding 0.9 ml of substrate mixture. Inhibition experiments of lysosomal triglyceride lipase were performed at pH 4.4 (acetate buffer, I = 0.1).

<table>
<thead>
<tr>
<th>Protamine concentration</th>
<th>Hydrolytic activity of different fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ug/ml preincubation</td>
<td>% maximal activity</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>175</td>
<td>98</td>
</tr>
<tr>
<td>375</td>
<td>99</td>
</tr>
<tr>
<td>750</td>
<td>19</td>
</tr>
<tr>
<td>1500</td>
<td>15</td>
</tr>
<tr>
<td>3000</td>
<td>15</td>
</tr>
<tr>
<td>6000</td>
<td>23</td>
</tr>
</tbody>
</table>

The concentration of heparin present in this fraction was 25 units per ml. As discussed under “Results,” heparin antagonizes the inhibitory effect of protamine.

Protamine required for inhibition was also related to the heparin concentration in the preincubation medium. The addition of 50 units of heparin per ml to hepatic perfusate raised the concentration of protamine required for inhibition from 1500 μg to 6000 μg per ml; 6000 μg of protamine per ml failed to inhibit enzymatic activity when 200 units heparin per ml was added to the preincubation medium.

Further Purification of Plasma Membrane Triglyceride Lipase—The plasma membrane triglyceride lipase was partially purified by chromatography on the heparin affinity column. This column did not bind any of the lipase released from the membranes by heparin; all of this activity emerged in the void volume. Therefore, advantage was taken of the observation that enzyme activity could be released from the membranes in the absence of heparin (Table IV). From 30 to 50% of the lipase activity released by heparin was obtained by incubation in glycine buffer alone. Up to 80% of this enzymatic activity was bound to the column. About half of this activity was subsequently eluted by increasing salt concentrations, the maximal activity being obtained at conductivity 32 mmo l (Fig. 4). This chromatographic step resulted in a 15- to 20-fold increase in specific activity (Table VIII). The degree of purification as observed by sodium dodecyl sulfate gel electrophoresis is illustrated in Fig. 5. Sodium dodecyl sulfate or urea was required for satisfactory separation of the plasma membrane proteins. The lipolytic activity of the purified fraction was destroyed by 5 min prior incubation at 4°C with either of these components, and it was not possible to locate the position of the enzyme protein on polyacrylamide gel.

The activity of the partially purified fraction was not enhanced by the addition of rat plasma (0.01 ml per ml of assay), which contains an activator of lipoprotein lipase (3).

Discussion

We have further characterized the triglyceride lipase released by heparin from the perfused rat liver and have attempted to identify its subcellular origin. The heparin-released hepatic

<table>
<thead>
<tr>
<th>Time period of preincubation</th>
<th>Hydrolytic activity of different fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>% maximal activity</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>74</td>
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<td>10</td>
<td>64</td>
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<td>15</td>
<td>70</td>
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<td>20</td>
<td>37</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
</tr>
</tbody>
</table>

TABLE VII

Effect of time of preincubation with protamine on inhibition of post-heparin liver perfusate and different cell fractions

We have recently used a column in which heparin has been covalently linked to agarose according to the method of Cuatrecasas (44). The enzyme released from plasma membranes was completely bound to this column and could subsequently be released by increasing salt concentration.
lipase has a pH optimum at 9.5, an apparent $K_m$ of 1.28 mM, and is inhibited by prior incubation with protamine and salt.

Of the hepatic cellular fractions studied, only the plasma membranes contained triglyceride lipase activity with a pH optimum of 9.5. The lipolytic activity in isolated plasma membranes proved to be readily displaced by heparin, more than 85% being released during 15 min incubation in the presence of very small amounts of this substance. The enzyme activity had the same pH optimum, the same response to inhibitors, and the same $K_m$ as the lipase released from the perfused liver.

When plasma membranes were incubated in the absence of heparin, only 25% of their total lipase activity was released into the medium. Addition of heparin to the enzyme released in this fashion did not increase its activity. The nonspecific release of lipase activity may be due to solubilization of some membrane proteins by the alkaline incubation medium.

The specific activity of lipase having a pH optimum at 9.5 was 9- to 10-fold higher in the plasma membranes than in liver homogenate and about 8-fold higher than that in cytosol and microsomes. The lipase activity in cytosol and microsomes was inhibited by NaCl and protamine. These inhibition studies, as well as other characterization of enzyme activity in different cell fractions, were carried out at pH 9.5, the pH optimum of lipase in liver perfusate. This pH is higher than the optimum observed in cytosol and microsomes, although at pH 9.5 activity in these two-cell fractions is more than 80% of the maximum.

We were unable to determine to what extent the lipase activity in cytosol and microsomes represented contamination with enzyme located in plasma membranes. We observed release of some activity when plasma membranes were incubated with the buffer (pH 7.4) used throughout the homogenization and fractionation procedure. On the other hand, if the activities of triglyceride lipase and 5'-nucleotidase in plasma membranes were not affected differently by the homogenization and fractionation procedure, it is not possible to attribute all of the lipolytic activity at pH 9.5 in cytosol and microsomes to enzyme originating in the plasma membranes.

Lipase activity in plasma membranes has been described by Higgins and Green (21) who prepared plasma membranes according to the method of Wallach and Ullrey (41), and by Guder et al. (16), who employed the method of Coleman (42) with omission of the final gradient step. The enzyme described by Higgins and Green had a pH optimum in the acid range and appeared to be activated by heparin. The activity found by Guder et al. had a pH optimum of 7.6 and was activated, but not shown to be released, by heparin.

Stoffel and Greten first reported that acid triglyceride lipase activity was mainly localized in lysosomes (13), and these results have been confirmed by other authors (14-17). The present studies show that acid lipase of lysosomal origin was inhibited less than 10% by sodium chloride or protamine concentrations that inhibited alkaline lipase activity in other subcellular fractions.
fractions by >75%. Our data differ from the results of Maha-
devan and Tappel (15) who found no effect of protamine on
lyosomal lipase activity but observed a 50% inhibition with 0.5
m sodium chloride. These differences may be related to their
use of unlabeled glycerol ester as substrate and a different emulsi-
fication procedure.

There have been conflicting reports on the existence of a tri-
glyceride lipase in mitochondria. Purification of a lipase from
rat liver mitochondria has recently been reported (19), although
the purity of the cell fractions was not established unequivocal-
lly. We observed no lipolytic activity in mitochondria, either in
the acid or alkaline range. These mitochondria had a high degree
of purity as established by marker enzymes and electron mi-
croscopy. It is conceivable that mitochondrial lipase activity
could have been inhibited by Triton WR-1339. It has been
shown, however, that this detergent is not taken up by hepatic
mitochondria (43). Our data are consistent with the results of
other authors who found that lipolytic activity in the light
mitochondrial fraction followed the distribution patterns of
marker enzymes of lysosomes rather than mitochondria (16).

Tissue lipases are considered to be of major importance for
the metabolism of plasma triglycerides. The existence of lipo-
lytic activity against chylomicrons in liver has first been dem-
crated by Korn (2). His criteria for defining lipoprotein
lipase rested, in part, on its activation by heparin and sensitivity
to protamine and salt inhibition, and partly on a requirement
for a serum component for its optimum activity. This serum
component has been lately identified as one of the apolipoproteins
for a serum component for its optimum activity. This serum
activity over that present in whole liver homogenate.

lysosomal lipase was the apparent lack of requirement for
heparin affinity chromatography for further purification. This
purification of a lipase from
the rat and lipo-
protein lipase in extracellular tissues. Furthermore, it is also
not possible to distinguish between them in a functional sense.
The plasma membrane enzyme appears to be the major com-
ponent of lipolytic activity released by heparin into hepatic
perfusate. These perfusates contain activity capable of hy-
drolyzing triglycerides in lipoproteins. It has been recently
suggested that “remnants” of lipoproteins are further metabo-
lized in the liver (55, 56). The liver may be the site of catabol-
isim of both low and high density lipoproteins (57, 58), which
contain lesser but significant amounts of triglyceride. Lipolytic
activity at the plasma membrane may conceivably facilitate the
further catabolism of lipoproteins by promoting their delipidation
prior to entry into the hepatic cells.

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REFERENCES
1. La Rosa, J. C., Levy, R. I., Windmueller, H. G. & Fred-
rickson, D. S. (1972) J. Lipid Res. 13, 350-363
37, 574-578
64, 185-193
Life Sci. 7, 441-447
404-411
Chem. 239, 385-391
14, 439-445
126, 185-187
156
Chem. 348, 1145-1150
2849-2854
Acta 157, 175-185
Acta 137, 497-517
246, 7139-7143
144, 211-220
241, 2801-2809
24. de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R.
& Appleman, F. (1955) Biochim. J. 60, 604-617
(deDuve, A. V. S. and Cameron, M. P., eds) p. 176, J. &
A. Churchill, Ltd., London
(1964) Biochim. Biophys. Acta 90, 126-145
49. Norris, B. & French, J. E. (1968) Quart. J. Exp. Physiol. 43, 180-188