Removal and Metabolism of Triglycerides by Perfused Liver

MARTIN RODBELL, ROBERT O. SCOW, AND SIDNEY S. CHERRICK

From the Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland

(Received for publication, July 5, 1963)

The removal of triglycerides from the circulation by the liver has been studied with chylomicrons and emulsions containing labeled fatty acids and glycerol (1–5). During the disappearance of circulating triglycerides, the ratio of labeled glycerol to labeled fatty acids was the same in liver triglycerides as in plasma triglycerides. From these observations, it was concluded that triglycerides are removed intact by the liver. In interpreting these studies, however, it was assumed that glycerol released by hydrolysis of circulating triglycerides is not reincorporated into hepatic triglycerides (6). Since it is difficult to assess the degree to which this may occur, a study of the uptake and metabolism of triglycerides by the perfused rat liver was carried out with another type of labeled compound, glyceryl tripalmitin-1-14C. Tripalmitin can be readily separated from triglycerides containing unsaturated fatty acids (7), and since saturated triglycerides are removed intact by the liver, it was possible to differentiate between unmetabolized substrate and newly formed triglycerides in both hepatic cells and blood.

EXPERIMENTAL PROCEDURE

Perfusion Procedures—Female Sprague-Dawley rats weighing 170 to 200 g were used in these experiments. They were fed a high fat diet for at least 1 week. The composition of the diet was: casein, 28%; sucrose, 21%; hydrogenated vegetable oil, 37%; cottonseed oil, 8%; whole liver powder, 5%; vitamins A, D, and E; and salts. Both liver and blood donors were fasted overnight.

The livers, which weighed 5.0 to 6.5 g, were perfused by the technique of Mortimore (8), in which the liver is left in the animal. The perfusing fluid was heparinized rat blood diluted 1:1 with 0.85% NaCl solution. The initial volume of the perfusing fluid was 66 ml. The liver was prepared for perfusion by anesthetizing the donor rat with ether and placing a ligature around the hepatic artery and another around the inferior vena cava between the liver and the right renal vein. The inflow cannula was inserted into the portal vein, and the outflow cannula, into the inferior vena cava above the diaphragm. Blood flow through the liver was re-established after an interval of less than 3 minutes. The rat was covered with plastic film (Saran Wrap, Dow Chemical Company) and placed with several feet of inflow tubing into an oven maintained at 38–39°. The blood was oxygenated in a rotating flask with a mixture of 95% O2-5% CO2 and filtered through Japanese silk screen (7 × 7). The blood was pumped up to the liver by a finger action pump and returned to the reservoir flask by gravity. Blood flow rate was 1.0 to 1.5 ml per g of liver each minute. Blood was circulated through polyethylene tubing.

Blood samples were taken during the perfusion from the outflow tube (hepatic vein); terminal samples were obtained from the oxygenator-reservoir.

14C Tripalmitin Emulsion—The 14C tripalmitin emulsion used in these studies was kindly supplied by the Upjohn Company. The emulsion (Lipomul) contained cottonseed oil, 15%; glucose, 4%; soybean lecithin, 2%; and pleuronic F68, 0.3% (weight per volume basis). Glyceryl tripalmitate-1-14C was mixed in trace amounts with cottonseed oil before preparation of the emulsion. The specific activity of the emulsion was 950 c.p.m. per μeq of triglyceride ester. The particle size of the emulsion, determined by passing a dilute suspension through Millipore filters (Millipore Corporation, New Bedford, Massachusetts) of different sizes, was between 0.1 and 0.45 μ.

The day before each experiment, 2 to 3 ml of the emulsion were dialyzed against 1 liter of 0.9% NaCl at 5° for 18 hours. The dialyzed emulsion was added to the blood and circulated through the perfusion apparatus for 40 minutes before perfusing the liver. The uptake of the dialyzed emulsion by the liver was approximately twice that of undialyzed emulsion (Table I). Addition of glucose had no effect on the uptake of the dialyzed emulsions. The increased uptake of the dialyzed emulsion may have been due to the removal of pleuronic F68 by dialysis, because a substance similar in structure, pleuronic F88, inhibits uptake of artificial emulsions injected in vivo (9).

Albumin-14C-Palmitate Complex—In 1 ml of 0.025 M NH4 at 60° were dissolved 2 to 3 mg of palmitic acid-1-14C, and 2 ml of a 1.5% solution of bovine serum albumin, adjusted to pH 7.4 with NH4, were added to the palmitate solution at 40° with stirring. Sufficient NaCl was then added to bring its concentration to 0.9%. The concentration of free fatty acids in the perfusion fluid after addition of the palmitate solution was 0.5 μeq per ml; the initial volume was 66 ml.

Analyses of Blood

Determination of Triglycerides—Samples of blood (1 ml) were added to 3 ml of Dole's isopropyl alcohol-heptane-H2SO4 reagent (10). After time was allowed for disruption of the red cells and flocculation of the proteins, 2 ml of water and 5 ml of heptane were added. Aliquots of the heptane phase were added directly to counting vials, dried under a stream of nitrogen at 40°, and counted as described below.

The triglyceride ester content in the heptane phase was deter-
Table I

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>No. of experiments</th>
<th>Initial blood glucose (mg/100 ml)</th>
<th>Mean uptake of triglycerides in 15 minutes (wet/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not dialyzed</td>
<td>2</td>
<td>88</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Dialyzed</td>
<td>6</td>
<td>31</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>Dialyzed + glucose</td>
<td>2</td>
<td>111</td>
<td>8.9 ± 0.9</td>
</tr>
</tbody>
</table>

*After addition of 0.7 ml of emulsion to 66 ml of diluted blood.
† Calculated on the basis of initial specific activity, 674 ± 50 c.p.m. per peq of ester. The initial concentration of triglyceride ester in blood was 6 to 9 peq per ml. Mean ± standard error.
‡ Glucose (56 mg) added to 66 ml of diluted blood.

Removal and Metabolism of Triglycerides by Perfused Liver

Vol. 239, No. 2

Lipid Extraction—At the end of each perfusion with $^{14}$C emulsion, the liver was perfused with 30 ml of 0.9% NaCl without recirculation to remove radioactivity from the blood vessels; this took about 5 minutes. The liver was then blotted on absorbent paper and weighed. The lipids were extracted by the method of Bligh and Dyer (17). The chloroform extract was dried over Na$_2$SO$_4$ and evaporated to dryness at 60° under nitrogen. The residue was dissolved in 5 ml of chloroform, which was passed through a 5-g silicic acid column (100 to 200 mesh). Elution of the neutral lipids was accomplished with 50 ml of chloroform. The phospholipids were subsequently eluted with 50 ml of methanol. Complete recovery of $^{14}$C and esters in the original chloroform extract was obtained in the two eluates. The ester and radioactivity contents of the liver lipids are expressed as micro equivalents of ester and counts per minute per g of liver, wet weight. The total radioactivity and the lipid content of portions of liver taken from different lobes were the same, indicating that the liver was perfused uniformly.

Isolation of Tripalmitin from Neutral Lipid Fraction—Tripalmitin, 25 μmoles recrystallized from heptane, was added to the neutral lipid fraction. After the chloroform was removed at 40° under vacuum, the residue was dissolved in 8 ml of ethanol-acetone (85:15) at 60° and stored overnight at 5°. The precipitate formed was collected on a sintered glass filter of medium porosity and washed twice with 5-ml portions of cold (5°) absolute ethanol. The filtrate and washings contained the neutral lipid fraction, and the precipitate, tripalmitin. The quantity of ester in the neutral lipids was not changed by addition and removal of carrier tripalmitin. The added carrier tripalmitin was quantitatively recovered in the ethanol-acetone-insoluble fraction. After three recrys tallizations from heptane at 5°, there was no change in the specific activity of tripalmitin. Gas-liquid chromatography showed that palmitic acid was the only fatty acid in the isolated tripalmitin fraction. The rat livers used in these studies did not contain, before perfusion, any triglycerides which were insoluble in cold alcohol-acetone.

Radioactivity Determination—Radioactivity was measured in a Packard Tri-Carb scintillation counter. The scintillator solution used for lipid and ketone bodies was 0.4% 2,5-diphenyloxazole in toluene. $^{14}$CO$_2$ was determined by the method of Steinberg (18). Appropriate corrections were made for background and quenching.
through three times, with the pore size decreased progressively from 1 mm to 0.1 mm. The reticuloendothelial cells which contained iron were separated from parenchymal cells by magnetic force and sedimentation. The reticuloendothelial cells were suspended in 0.9% NaCl with 0.01 M Tris buffer, pH 7.4, and redispersed by passage through silk screen (7 xs). Aliquots were taken for lipid ester and micro-Kjeldahl nitrogen analysis. Histological examination showed that the reticuloendothelial cell fraction was homogeneous and that the parenchymal cell fraction contained practically no iron.

Cell Fractionation Studies—A 10% liver homogenate was prepared in isotonic sucrose, and the cellular components were separated by the method of Hogeboom (20). The analytical procedures outlined in the previous sections were carried out on nuclear and cell debris, mitochondrial, microsomal, and supernatant fat fractions. Based on measurements of phospholipid phosphorus and neutral lipid ester, 90 to 100% of the hepatic lipids were recovered. The phospholipid content (P:N ratio) in mitochondrial and cell debris fractions did not change when the fractions were layered over 0.25 M sucrose and recentrifuged.

RESULTS

Removal of 14C-14C-Triglycerides as Function of Concentration—The rate of removal of 14C-triglycerides from blood by the perfused liver is shown in Fig. 1. Approximately 75% of the 14C-lipid was removed within 60 minutes when the initial blood concentration of triglyceride ester was 2.4 μeq per ml; less than 20% was removed when the concentration was 8.0 to 8.3 μeq per ml. Disappearance of 14C-lipid during the second hour was negligible at both concentrations.

The effect of the initial concentration on the change in specific activity of blood triglycerides is shown in Fig. 2. The change during 2 hours of perfusion is expressed as the ratio of the initial to the final specific activity. At concentrations less than 4 μeq of triglyceride ester per ml, the specific activity of the circulating triglycerides decreased 50 to 75%, whereas above 7 μeq per ml it decreased only 10 to 15%.

Since the triglycerides in rat blood and in the emulsion (cottonseed oil) differ from tripalmitin, a study was made to determine whether there was selective removal of tripalmitin by the liver. The data in Table II show that this did not occur. The amount of 14C-tripalmitin removed was proportional to the concentration of triglyceride ester, not label, in the blood.

Removal of 14C-Palmitate from Blood—The decrease in the specific activity of the circulating triglycerides during the perfusion suggested that triglycerides of the liver were entering the blood. Triglyceride secretion was investigated by perfusing livers with blood containing 14C-palmitate (Experiment A) (Fig. 3). The blood triglyceride concentration in one experiment was increased 5-fold by adding unlabeled emulsion (Experiment B). The disappearance of 14C-palmitate from the perfusate in both experiments was essentially the same, and 4% of the initial radioactivity appeared in blood triglycerides within 30 minutes. Accumulation of radioactivity in circulating triglycerides during the next 90 minutes, however, was doubled when the emulsion was added to the blood. This difference could be the result of dilution in the blood of the secreted 14C-triglycerides, which would have reduced their recycling through the liver.

The histological examinations were made by Dr. Mearl Stanton, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
Table II
Effect of blood triglyceride concentration on removal of 1\(^{14}\)C-tripalmitin by perfused liver

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Initial blood triglycerides μeq ester/ml</th>
<th>Specific activity of blood triglycerides c.p.m./μeq ester</th>
<th>(^{14})C-Triglyceride in blood after 2 hours % initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3</td>
<td>281</td>
<td>83</td>
</tr>
<tr>
<td>9</td>
<td>8.9</td>
<td>227</td>
<td>179</td>
</tr>
<tr>
<td>8</td>
<td>8.3</td>
<td>628</td>
<td>552</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of blood triglyceride concentration on removal of 1\(^{14}\)C-palmitate and release of 1\(^{14}\)C-triglycerides by the perfused liver. Initial blood triglyceride concentration: Experiment A (○, ●), 1.2 μeq per ml; Experiment B (△, ▲), 7.0 μeq per ml. Initial plasma free fatty acid concentration was 0.5 μeq per ml for both experiments.

Table III
Distribution of 1\(^{14}\)C in liver and blood after 2-hour perfusion with 1\(^{14}\)C-palmitate or 1\(^{14}\)C-tripalmitin

<table>
<thead>
<tr>
<th>Added to blood</th>
<th>Blood triglyceride ester μeq/ml</th>
<th>Recovered in liver %</th>
<th>Recovered in perfusate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{14})C-Tripalmitin emulsion</td>
<td>12</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>1(^{14})C-Palmitate + emulsion</td>
<td>7</td>
<td>98</td>
<td>18</td>
</tr>
<tr>
<td>1(^{14})C-Palmitate</td>
<td>1.2</td>
<td>98</td>
<td>7</td>
</tr>
</tbody>
</table>

* Triglycerides other than tripalmitin. Unlabeled tripalmitin was added to neutral lipid fraction and dissolved in ethanol-acetone (85:15). Tripalmitin precipitated at 5°C and was filtered. Neutral lipids in the filtrate were fractionated on a silicic acid column into cholesterol esters and triglycerides by the method of Horning, Williams, and Horning (21). All of the radioactivity in the filtrate was recovered in the triglyceride fraction.

† The initial concentration of free fatty acids was 0.5 μeq per ml.

‡ Unlabeled emulsion.

The relative distribution of radioactivity between phospholipid and triglyceride fractions in the liver was the same whether the liver was perfused with 1\(^{14}\)C-tripalmitin or 1\(^{14}\)C-palmitate. Based on the total amount of 1\(^{14}\)C-lipids found in blood and in the liver, more radioactivity was incorporated into neutral lipids than phospholipids. With both substrates, the radioactive fatty acid in neutral lipids and phospholipids was located primarily in the α position.²

² M. Rodbell, unpublished data.
TABLE IV
Relationship between concentration of triglycerides in blood and amount removed by liver during 2 hours of perfusion

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Initial blood triglycerides</th>
<th>Decrease in ester content of blood</th>
<th>Total 14C-lipids in liver</th>
<th>μeq ester/g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.4</td>
<td>413</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>6.8</td>
<td>770</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>8.3</td>
<td>628</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>9</td>
<td>8.9</td>
<td>227</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>12.0</td>
<td>806</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

* Calculated from the initial specific activity of the blood triglycerides and the 14C content of the hepatic lipids.

TABLE V
Lipid content and distribution of radioactivity in liver lipids after 2-hour perfusion with 14C-tripalmitin emulsion

Same experiments are reported in Table IV.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Blood triglycerides</th>
<th>Neutral lipids</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14C-Neutral lipid</td>
<td>14C-Total</td>
<td>14C-Phospholipid</td>
</tr>
<tr>
<td></td>
<td>μeq ester/ml</td>
<td>μeq ester/g liver</td>
<td>μeq ester/g liver</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>111</td>
<td>7.3</td>
</tr>
<tr>
<td>5</td>
<td>5.5</td>
<td>45</td>
<td>6.0</td>
</tr>
<tr>
<td>8</td>
<td>5.4</td>
<td>79</td>
<td>5.5</td>
</tr>
<tr>
<td>9</td>
<td>5.8</td>
<td>59</td>
<td>8.4</td>
</tr>
<tr>
<td>11</td>
<td>12.0</td>
<td>66</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Mean ± s.e. 5.6 ± 0.3 78 ± 18.6 ± 1.162 ± 1

* Calculated from the initial specific activity of blood triglycerides and the 14C content of each fraction (See Table IV).
† Calculated from the initial specific activity of blood triglycerides and 14C content of tripalmitin fraction.
‡ Derived from ester analyses on fractions from silicic acid columns.

Distribution of 14C-Lipids in Liver at Different Times—The livers were perfused with diluted blood for 20 minutes and then with fresh blood containing the labeled emulsion, 7.4 μeq of ester per ml, for 5, 15, 30, or 60 minutes. The livers were then flushed with 30 ml of 0.9% NaCl solution for 5 minutes to remove all radioactivity from the blood vessels. The amounts of 14C-neutral lipid, 14C-phospholipids, and unmetabolized 14C-triglyceride in the liver were calculated from the 14C content of each fraction and the initial specific activity of the blood triglycerides. The values recorded in Fig. 4 are expressed as microequivalents of ester per g of liver.

The amount of unmetabolized 14C-triglycerides in the liver was essentially the same at all time points. This would suggest that the site of entry of triglycerides were rapidly saturated and remained so throughout 60 minutes of perfusion. Accumulation of 14C in the neutral lipid and phospholipid fractions, however, increased with time and was reflected by the increase in total radioactivity in the liver. As also seen in other experiments (Table V), the neutral lipid and phospholipid fractions in the liver contained similar amounts of 14C.

Distribution of 14C-Lipids in Subcellular Fractions of Liver—The intracellular distribution of different 14C-lipids was determined in livers perfused for 15 and 120 minutes. The livers were homogenized and separated into the following fractions: nuclei and cell debris, mitochondria, microsomes, and supernatant fat. After 15 minutes of perfusion, 79% of the 14C-lipids in the liver were present as unmetabolized triglycerides in the supernatant fat fraction (Table VI). The microsomal fraction contained 12% of the 14C-lipids, all as neutral lipids and phospholipids, whereas the mitochondrial and nuclear and cell debris fractions contained very little radioactivity at 15 minutes. Eighty per cent of the unlabel neutral lipid esters were recovered in the supernatant fat fraction (Table VI). The phospholipids were...
TABLE VI

Distribution of 3H-lipids in subcellular fractions of liver after 15 minutes of perfusion

Results are based on the average of three experiments.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Ester content</th>
<th>% total</th>
<th>3H content</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral lipid</td>
<td>Phos-</td>
<td>Unmetabol-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pholipid</td>
<td>ized triglyceride</td>
<td></td>
</tr>
<tr>
<td>Nuclei and cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>debris</td>
<td>4</td>
<td>21</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>2.5</td>
<td>10.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Microsomes</td>
<td>3.5</td>
<td>18</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Supernatant fat</td>
<td>40</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
* Tripalmitin.

TABLE VII

Distribution of 14C-tripalmitin and metabolites in parenchymal and reticuloendothelial cells of liver

Results are the average of four experiments ± standard error. Livers from iron-treated rats (see "Experimental Procedure" for details) were perfused for 15 minutes with blood which contained 7 µg of triglyceride ester per ml (specific activity, about 650 c.p.m. per µg). Liver cells were separated into reticuloendothelial cells and parenchymal cells as described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Cell preparation</th>
<th>Total 14C-lipid</th>
<th>14C content</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m./mg N</td>
<td>Neutral lipid</td>
<td>Phos-</td>
<td>Tripalmitin</td>
<td></td>
</tr>
<tr>
<td>Whole liver*</td>
<td>100</td>
<td>33 ± 2</td>
<td>30 ± 3</td>
<td>38 ± 2</td>
<td></td>
</tr>
<tr>
<td>Parenchymal cells</td>
<td>100</td>
<td>31 ± 2</td>
<td>25 ± 2</td>
<td>44 ± 5</td>
<td></td>
</tr>
<tr>
<td>Reticuloendothelial</td>
<td>118</td>
<td>44 ± 4</td>
<td>38 ± 3</td>
<td>19 ± 2</td>
<td></td>
</tr>
</tbody>
</table>
* Distribution of 14C-lipids in whole liver, expressed as micro- equivalents of ester per g of liver, was: neutral lipid, 1.4; phospholipid, 1.2; tripalmitin, 1.6. Total 14C-lipid in whole liver was 4.2 µeq of ester per g of liver.

found principally in the microsomal and cell debris fractions and, to a lesser extent, in the mitochondrial fraction.

In one liver perfused for 120 minutes, the unmetabolized 14C-triglyceride was found primarily in the supernatant fat fraction. The 14C-phospholipids (44% of total) were concentrated only in the particulate fractions, whereas the 14C-neutral lipids (56% of total) were present in all fractions. Approximately 10% of the radioactivity in the liver was found as neutral lipids in the supernatant.

Distribution of 14C-Lipids in Parenchymal and Reticuloendothelial Cells of the Liver—The preceding observations showed that the liver removed 14C-triglycerides intact from blood, and about two-thirds were metabolized to phospholipids and neutral lipids within 2 hours (See Tables III and V). Since the liver is composed of two principal types of cells, parenchymal and reticuloendothelial, the role of each cell type in removal and metabolism of the 14C emulsion was investigated.

Livers from iron-treated rats were perfused with the labeled emulsion for 15 minutes and then separated into two fractions, iron-containing reticuloendothelial cells and parenchymal cells. The total uptake of 14C-triglyceride by the iron-treated livers was 60% of that taken up in 15 minutes by normal livers (Table VII; Fig. 4).

The concentration of 14C-lipid (counts per minute per mg of nitrogen) was the same in reticuloendothelial and parenchymal cell fractions in the whole liver (Table VII). Distribution of radioactivity in the two cell fractions, however, was different. In parenchymal cells, 31% of the radioactivity was recovered as neutral lipid, 35% as phospholipids, and 44% as unmetabolized triglycerides. In reticuloendothelial cells, 44% was present as neutral lipid, 38% as phospholipids, and 19% as unmetabolized triglycerides.

DISCUSSION

The labeled triglyceride, glyceryl tripalmitin-1-14C, was removed from the blood and metabolized by the liver in the same manner as the other triglycerides in the emulsion. The amount of unmetabolized triglycerides found in the liver after 5 minutes, 6 µeq of triglyceride ester per g of liver, was the same as that after 60 minutes of perfusion. This suggests that the entry sites were saturated within 5 minutes. The turnover time of this amount of triglyceride was 40 minutes, but this is probably an overestimate, since only the 14C-lipid remaining in the liver was considered. It is apparent that the initial uptake was considerably faster than the transfer of triglyceride fatty acids from entry sites to other parts of the cells for metabolism. This could explain the inverse relationship between the fractional removal of 14C-triglycerides and the blood triglyceride concentration seen in Fig. 1 and observed by others in studies in vivo (21).

A comparison was made of the metabolism of plasma free fatty acids and triglycerides by the perfused liver. The equal distribution of radioactivity in the neutral lipid and phospholipid fractions when either 14C-tripalmitin-labeled emulsion or 14C-palmitate was perfused indicated that both substrates followed similar metabolic pathways. However, a larger fraction of radioactivity was found in ketone bodies when 14C-palmitate was perfused. Neither uptake nor metabolism of 14C-palmitate was affected by addition of unlabelled triglycerides. A large fraction of the labeled neutral lipids formed in the liver was transferred to the blood, indicating that secretion is an important factor in the turnover of triglycerides by the liver (23).

The subcellular fractionation studies did not reveal the nature of the intracellular structures responsible for the uptake of triglycerides by the liver. However, they may have been destroyed by the fractionation procedure or included in the supernatant fat fraction. The large percentage of label found in neutral lipids and phospholipids in the microsomal fraction is probably related to the proposed role of the endoplasmic reticulum in the formation and secretion of lipoproteins by the liver (24, 25).

14C-Tripalmitin was found in parenchymal and reticuloendothelial cells. The preceding observations showed that triglycerides are removed from blood by both types of cells without de-esterification. It is evident from the data in Table VII that both cell types converted blood triglycerides to phospholipids and neutral lipids. A quantitative evaluation of their role in the removal and metabolism of blood triglycerides is difficult, because the iron treatment used for separation of the cells reduced the uptake of triglyceride by the perfused liver. It could not be determined whether the
iron treatment affected the reticuloendothelial or both types of cells.
Di Luzio and Reggi (26, 28) have reported that chylomicrons are removed exclusively by the parenchymal cells, whereas artificial fat emulsions (Lipomul) are taken up by reticuloendothelial cells of the liver. In our study the emulsion (Lipomul) was modified by dialysis (which increased triglyceride uptake by the liver) and by incubating the emulsion, after dialysis, with blood for 40 minutes before perfusion. The latter should be expected to convert part of the emulsion to chylomicron-like particles. It is likely, therefore, that both emulsion and chylomicron-like particles were present in the perfusion fluid and that this accounts for the presence of unmetabolized triglycerides in both cell types.

SUMMARY
The metabolism of a triglyceride emulsion labeled with glyceryl tripalmitin-1,4C was studied in the perfused rat liver. Tripalmitin was removed and metabolized by the liver in the same manner as the other triglyceride present in the emulsion. The use of tripalmitin made it possible to distinguish between the unmetabolized substrate and newly formed triglycerides in the liver and blood.

The fractional disappearance of 14C-lipid from blood was inversely related to the blood triglyceride concentration. The entry sites in the liver were saturated within 5 minutes. At this time, 90% of the 14C in the liver was tripalmitin, indicating that triglycerides are removed from the blood without de-esterification. At 15 minutes, 80% of the radioactivity in the liver was found as tripalmitin in the supernatant fat fraction of liver homogenates, and 12% as neutral lipid and phospholipids in the microsomal fraction. During a 2-hour perfusion, half of the radioactivity removed by the liver was secreted to the blood, primarily as triglycerides; the other half remained in the liver as neutral lipid, phospholipid, and tripalmitin.

The distribution of radioactive lipids between parenchymal and reticuloendothelial cells was measured in livers of rats treated with carbonyl iron. The uptake of tripalmitin by these livers was 60% of that found in untreated livers. 14C-Tripalmitin and its metabolites were found in parenchymal and reticuloendothelial cells, which indicates that both types of cells participate in the removal and metabolism of the triglyceride emulsion.

REFERENCES