The Action of Carbon Tetrachloride on the Transport and Metabolism of Triglycerides and Fatty Acids by the Isolated Perfused Rat Liver and Its Relationship to the Etiology of Fatty Liver*

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The induction of a fatty liver and the generalized hepatotoxicity associated with chlorinated hydrocarbons, such as carbon tetrachloride and chloroform, is well known (1, 2). It has been demonstrated recently that the administration of CC\textsubscript{4} to an animal produces a marked increase in hepatic triglyceride levels (3, 4). Clearly, such an increase could be the result of increased synthesis of triglycerides, decreased oxidation, increased uptake of triglycerides or fatty acids from the blood, decreased secretion of triglycerides by the liver, or any combination of these factors.

We have investigated these aspects of triglyceride transport and metabolism in the isolated perfused rat liver. It appears from our observations that the major metabolic defect induced by CC\textsubscript{4} intoxication is an inhibition of hepatic triglyceride release. This inhibition of outward transport would allow the accumulation of triglycerides within the liver and the occurrence of the syndrome of CC\textsubscript{4} fatty liver. Preliminary reports of this work have appeared (5, 6).

**EXPERIMENTAL PROCEDURE**

Male Sprague-Dawley rats weighing 250 to 400 g were maintained on a balanced ration and given water *ad libitum*. Animals serving as liver donors were lightly anesthetized with ether and given a CCl\textsubscript{4}-mineral oil mixture (1:1 by volume), 0.5 ml per 100 g of body weight, by gastric intubation. The liver was isolated 3½ hours after CCl\textsubscript{4} administration and perfused through the portal vein *in vitro*. The control groups received either an equivalent amount of mineral oil or no treatment at all. Blood for the perfusion medium was obtained by aortic puncture from normal fed male rats under ether anesthesia. Details of the perfusion procedure and analytical methods have been reported previously (7). Total ketones were estimated by the method of Michaels et al. (8) after oxidation in the apparatus described by Greenberg and Lester (9). Total lipid-soluble phosphorus was determined by the procedure of King (10) on aliquots of the methanol fractions obtained after elution of lipids chromatographed on silicic acid. Triglycerides were estimated by the method of Van Handel and Zilversmit (11) after adsorption of phospholipids on silicic acid. Unless otherwise specified, the total amounts of mono-, di-, and triglycerides were determined by our routine procedure and calculated as triglyceride. Radioactivity was measured by liquid scintillation counting techniques (7).

**RESULTS**

**Release of Triglyceride by Perfused Liver**—As reported previously, a net increase of the triglyceride concentration of the medium was observed during perfusion of rat liver isolated from a normal fed animal (7). In contrast, there was no net release of triglyceride by the liver from a CCl\textsubscript{4}-poisoned rat (Fig. 1). There was, indeed, uptake of the small amount of endogenous triglyceride initially present in the perfusate. Although the addition of palmitate to the medium increased the net release of triglyceride by the liver from a normal fed animal, the fatty acid failed to affect triglyceride release by livers from CCl\textsubscript{4}-treated rats. It is of interest that the apparent inhibition of triglyceride release by the liver from a CCl\textsubscript{4}-poisoned rat occurred in the presence of elevated hepatic triglyceride levels (Table II). The perfusate phospholipid concentration paralleled triglyceride changes (Fig. 2). Quantitatively, however, these changes were relatively minor in relation to the liver phospholipid content (Table II).

**Uptake of Triglycerides and Fatty Acids by Perfused Liver**—The uptake of nonesterified fatty acid as the palmitate-rat serum complex was quite rapid and occurred at apparently identical rates in livers from both normal and CCl\textsubscript{4}-poisoned rats (Fig. 3). Minimal all of the palmitate was removed by the liver within 30 minutes after addition to the perfusate. The stimulation of triglyceride secretion induced by the addition of the palmitate was, however, maintained for the duration of the experiment (Fig. 1). There was no significant difference in the rate of uptake of a tripalmitin-1-C\textsuperscript{14}-labeled neutral fat emulsion between livers from CCl\textsubscript{4}-poisoned animals and those from non-treated animals. The uptake of the emulsion by livers from animals that had received mineral oil without CCl\textsubscript{4} was, surprisingly, more rapid than the uptake by livers from untreated animals (Fig. 4).

The measurement of the disappearance of tripalmitin radioactivity from the medium is a more accurate estimate of triglyceride uptake by the livers from fed animals than is chemical determination of perfusate triglycerides. Interpretation of the
Acetate Incorporation into Liver Lipids—Livers were perfused with a medium containing acetate-1-\(^{14}\)C. Two hours later the liver was removed from the system and perfused with ice-cold 0.9% NaCl to remove any residual blood-buffer perfusate. Total lipids were isolated from the liver by extraction with alcohol, ether, and petroleum ether (12). In preliminary experiments, hepatic lipids were separated on silicic acid into a chloroform eluate fraction containing triglycerides, as well as other lipid components (F1), and a methanol eluate fraction containing phospholipids (F2). The results are presented in Tables I and II. Livers from CCL-poisoned animals incorporated more total counts from acetate-1-\(^{14}\)C into the total liver lipids and F1 than did livers from normal fed animals. Approximately 60% of the incorporated counts were found in the triglyceride-containing F1, and 40% in the phospholipid fraction, whereas with the control animals the reverse was observed. The increased incorporation of acetate-1-\(^{14}\)C into the F1 fraction of livers from CCL-treated rats was accompanied by a simultaneous increase of the chemically estimated hepatic triglyceride. No change in total hepatic lipid-soluble phosphorus or counts incorporated into the F2 fraction was induced by CCL administration. In order to ascertain whether the increase in hepatic triglyceride concentration which follows CCL poisoning was due to increased synthesis de novo, as suggested by the increased incorporation of acetate-1-\(^{14}\)C into the triglyceride-containing F1 fraction, liver
Lipids were separated by silica acid chromatography on a Hirsch-Ahrens column (13) with the solvent mixtures described by Horning, Williams, and Horning (14). Column effluent fractions were monitored by thin layer silica acid chromatographic procedures (15). The specific radioactivity of the purified triglycerides is presented in Table I. Although the mineral oil per se apparently stimulated triglyceride synthesis, the simultaneous administration of CCl₄ and mineral oil did not. One may surmise from these observations that the increase in counts in the hepatic triglycerides from CCl₄-poisoned rats after perfusion with acetate-1-⁴¹C probably reflects the increase in liver triglyceride pool size resulting from a block in outward triglyceride transport.

Ketone body production by the perfused liver was not affected by the administration of CCl₄ to the liver donor, regardless of the presence or absence of added lipid substrate (Fig. 5). Curiously, the mineral oil alone slightly depressed ketone body production in the absence of added lipid substrate.

In the absence of exogenous lipid substrate, or in the presence of added palmitate, more urea was produced by livers from normal rats than by those from CCl₄-poisoned animals (Fig. 6). The addition of a synthetic neutral fat emulsion or of washed rat chylomicra (7) depressed urea formation by the liver. Under these conditions, there was no further significant depression of urea formation by CCl₄ administration.

The glucose concentration of the perfusate is maintained by the liver during the course of the perfusion (Fig. 7). Carbon tetrachloride poisoning depressed glucose release by livers from

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**Table I**

Lipid synthesis from acetate-1-⁴¹C by isolated perfused liver

Livers were perfused with the blood-buffer medium. Initial volume was 100 ml, to which 5.0 mg of acetate-1-⁴¹C (0.27 μC per mg) were added after a 20-minute equilibration period. Results are expressed as means ± standard deviations. Statistical analysis (CCl₄ versus normal): for A, t = 1.95, p = 0.042; for B2, t = 9.55, p < 0.0001; for C2, t = 7.90, p < 0.0001.

<table>
<thead>
<tr>
<th></th>
<th>CCl₄ (six experiments)</th>
<th>Normal (seven experiments)</th>
<th>Mineral oil control (four experiments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Per cent of administered counts per minute from acetate-1-⁴¹C incorporated per g of liver (dry) into total liver lipids</td>
<td>2.08 ± 1.19</td>
<td>2.03 ± 0.52</td>
<td>1.98 ± 0.57</td>
</tr>
<tr>
<td>B. 1. Per cent of administered counts per minute from acetate-1-⁴¹C incorporated per g of liver into triglyceride fraction, F₁</td>
<td>1.95 ± 0.74</td>
<td>0.81 ± 0.26</td>
<td>0.80 ± 0.32</td>
</tr>
<tr>
<td>2. Per cent of liver lipid counts in triglyceride fraction, F₁</td>
<td>65.4 ± 4.7</td>
<td>39.7 ± 5.0</td>
<td>39.7 ± 6.3</td>
</tr>
<tr>
<td>C. 1. Per cent of administered counts per minute from acetate-1-⁴¹C incorporated per g of liver into phospholipid fraction, F₂</td>
<td>1.05 ± 0.39</td>
<td>1.22 ± 0.33</td>
<td>1.15 ± 0.29</td>
</tr>
<tr>
<td>2. Per cent of liver lipid counts in phospholipid fraction, F₂</td>
<td>35.5 ± 5.4</td>
<td>60.0 ± 5.8</td>
<td>59.0 ± 4.9</td>
</tr>
<tr>
<td>D. Counts per minute per pmole of triglyceride (purified)</td>
<td>170 ± 130</td>
<td>148 ± 480</td>
<td>367 ± 485</td>
</tr>
</tbody>
</table>

**Table II**

Lipid content of perfused livers

The data were obtained from the experiment described in Table I. Results are expressed as means ± standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>CCl₄ (six experiments)</th>
<th>Normal (seven experiments)</th>
<th>Mineral oil control (four experiments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Liver weight, wet (grams)</td>
<td>10.36 ± 2.77</td>
<td>12.50 ± 2.52</td>
<td>11.76 ± 2.52</td>
</tr>
<tr>
<td>B. Liver weight, fat free, dry (grams)</td>
<td>10.36 ± 2.77</td>
<td>12.50 ± 2.52</td>
<td>11.76 ± 2.52</td>
</tr>
<tr>
<td>C. Total liver triglyceride (micromoles)</td>
<td>174.00 ± 50.45</td>
<td>78.90 ± 12.65</td>
<td>94.91 ± 12.65</td>
</tr>
<tr>
<td>D. Total liver lipid-soluble P (milligrams)</td>
<td>13.83 ± 50.45</td>
<td>14.86 ± 12.65</td>
<td>14.06 ± 12.65</td>
</tr>
<tr>
<td>E. Total liver lipids (grams)</td>
<td>0.6261 ± 1.29</td>
<td>0.6520 ± 1.29</td>
<td>0.6520 ± 1.29</td>
</tr>
</tbody>
</table>
HOURS

FIG. 5. Net ketone body production by liver. Composition of perfusate with added nonesterified fatty acid (NEFA) is that described for Fig. 3; with added tripalmitin-1-\(^{14}\)C-labeled synthetic emulsion (NFE), refer to Fig. 4. A, Means ± s.d., 3-hour data: 1 (normal) = 1.03 ± 0.24; 2 (mineral oil) = 0.63 ± 0.21; 3 (CCl\(_4\)) = 0.58 ± 0.32. (1 versus 2: \(t = 2.33; p = 0.031\). 1 versus 3: \(t = 2.32; p = 0.028\). 2 versus 3: \(t = 0.27; p = 1.00\).

B, Means ± s.d., 1-hour data: 1 (normal) = 0.43 ± 0.21; 2 (mineral oil) = 0.39 ± 0.10; 3 (CCl\(_4\)) = 0.53 ± 0.25. (1 versus 2: \(t = 0.35; p = 0.50\). 1 versus 3: \(t = 0.66; p = 0.33\). 2 versus 3: \(t = 1.06; p = 0.20\).

C, Means ± s.d., 3-hour data: 1 (normal) = 0.98 ± 0.42; 2 (CCl\(_4\)) = 0.96 ± 0.39.

HOURS

FIG. 6. Net urea production by liver. Composition of perfusate with added NEFA is that described for Fig. 3; with added NFE, refer to Fig. 4. A, Means ± s.d., 2-hour data: 1 (normal) = 0.61 ± 0.05; 2 (mineral oil) = 0.63 ± 0.13; 3 (CCl\(_4\)) = 0.40 ± 0.14. (1 versus 2: \(t = 0.35; p = 0.50\). 1 versus 3: \(t = 3.57; p = 0.003\). 2 versus 3: \(t = 4.01; p = 0.0004\).)

B, Means ± s.d., 2-hour data: 1 (normal) = 0.39 ± 0.12; 2 (mineral oil) = 0.41 ± 0.11; 3 (CCl\(_4\)) = 0.32 ± 0.09. (1 versus 2: \(t = 0.29; p = 1.00\). 1 versus 3: \(t = 3.57; p = 0.017\). 2 versus 3: \(t = 1.84; p = 0.06\).

C, Means ± s.d., 2-hour data: 1 (normal) = 0.61 ± 0.02; 

1 = 0.35 ± 0.02.

HOURS

FIG. 7. Perfusate glucose concentration. Perfusate did not contain any added lipid substrate. Means ± s.d., 2-hour data: 1 (normal) = 247 ± 51; 2 (mineral oil) = 199 ± 47; 3 (CCl\(_4\)) = 155 ± 60. (1 versus 2: \(t = 1.92; p = 0.042\). 1 versus 3: \(t = 4.31; p = 0.0003\). 2 versus 3: \(t = 3.37; p = 0.0014\).

DISCUSSION

A net increase in perfusate triglycerides was observed when livers from untreated normal fed rats or from those receiving mineral oil only were perfused with the blood-buffer medium, but not when livers from CCl\(_4\)-poisoned rats were employed. This is remarkable in view of the elevated hepatic triglyceride levels resulting from CCl\(_4\) intoxication. The inference is clear that the hepatic release of triglycerides may not only depend on the concentration of triglycerides within the cell, but may also be affected by chemical influences on the hepatic cell. Additional support for this conclusion is provided by the observation that livers of rats deprived of food for 48 hours did not show a net release of triglycerides as did livers from fed animals, even though the concentration of triglycerides in the livers from the unfed animals was equal to or greater than that from the fed rats (7). Failure to produce a net increase in perfusate triglycerides does not necessarily imply an absolute inhibition of release. Rather, the release of triglycerides relative to uptake may be slower in livers from CCl\(_4\)-poisoned rats than in livers from control animals. This is suggested by the rapid decrease in relative specific activity of the perfusate triglycerides when livers from control rats are perfused with a tripalmitin-1-\(^{14}\)C-labeled neutral fat emulsion, as compared to the relatively slow change when livers from CCl\(_4\)-poisoned animals are perfused.

It is improbable that the marked increase in hepatic triglyceride concentration, which is apparent within 1 hour after CCl\(_4\) administration, can be explained by decreased oxidation and subsequent accumulation of triglycerides. The oxidation of lipids by the liver of a CCl\(_4\)-poisoned rat appears to be within normal limits. Ketone body formation, one index of hepatic oxidation of fatty acids, was not depressed after CCl\(_4\) intoxication. Furthermore, the oxidation of octanoate by liver mitochondria in vitro is not impaired for at least the first 2 hours after CCl\(_4\) administration (18). The activation of fatty acids, which is a requirement for synthesis of triglycerides (19) and for the oxida-
tion of fatty acids (20), is not inhibited to any significant extent until the 5th hour after CCl₄ administration to the animal (21). The oxidation of octanoate and various tricarboxylic acid cycle intermediates by rat liver mitochondria was reported to be inhibited after CCl₄ poisoning, but these mitochondrial effects were not prominent until 10 to 15 hours after CCl₄ was administered to the animal (22). The mitochondrial oxidation rates could be restored to normal levels by the addition of pyridine nucleotides. The late loss of pyridine nucleotides (22, 23) and of adenosine polyphosphates (24) from liver mitochondria, the decreased oxidation of octanoate (22), and the uncoupling of oxidative phosphorylation (25) in liver mitochondria from CCl₄-poisoned rats may be consequences of mitochondrial membrane damage, but probably have no immediate relationship to the early accumulation of triglycerides within the hepatic cell.

The inhibition of outward transport of hepatic triglycerides would appear to be the major metabolic deficit induced by CCl₄ poisoning. Similar conclusions were reached by Schotz (29) on the basis of his studies with intact rats. A significant increase in hepatic triglyceride concentration is seen within 1 hour after CCl₄ poisoning (4). The concentration of CCl₄ in the liver is at a maximum within 1½ hours after administration (27). One of the earliest morphological changes seen in the hepatic cell within the first few hours after CCl₄ intoxication is swelling and disruption of the endoplasmic reticulum (16, 17). Mitochondrial damage is not evident at this time (17, 18). If the block in outward transport of triglycerides, with the resultant accumulation of triglycerides in the liver, is a sequence of injury to the endoplasmic reticulum, it would be reasonable to assume that some metabolic function associated with the endoplasmic reticulum is an absolute requirement for outward transport. Since uptake of triglycerides from the neutral fat emulsion continues at normal rates after the administration of CCl₄, it follows that triglycerides are transported in and out by separate metabolic pathways.

It was reported recently that the incorporation of glycine-l-C¹⁴ into the plasma proteins, fibrinogen and albumin, both of which are derived from the liver, is depressed after CCl₄ poisoning (4). Furthermore, glycine-l-C¹⁴ incorporation into mitochondrial, microsomal, and supernatant fractions of rat liver was diminished after CCl₄ administration (29). Protein synthesis has been shown to occur in microsomes and in smaller particles believed to be derived from the endoplasmic reticulum (30). It is possible that the outward transport of liver triglycerides depends on an adequate synthesis of lipoprotein. As a reasonable hypothesis for the mechanism of outward transport of hepatic triglycerides, it is suggested that synthesis of lipoprotein occurs at the endoplasmic reticulum from triglycerides synthesized at another cellular site. It is further suggested that triglycerides cannot be released by the liver except as a lipoprotein complex. Impairment of lipoprotein synthesis and, consequently, of outward hepatic triglyceride transport, without any significant simultaneous stimulation of triglyceride synthesis or any apparent inhibition of the uptake and oxidation of fatty acids and triglycerides, may be the etiology of the fatty liver of CCl₄ intoxication.

SUMMARY

A major metabolic defect induced by CCl₄ administration to the rat appears to be inhibition of the outward transport of hepatic triglycerides. This inhibition of outward triglyceride transport may be the etiology of the fatty liver associated with CCl₄ poisoning. Triglycerides were released into the medium by the isolated perfused liver obtained from normal fed rats. When livers were obtained from animals 3½ hours after CCl₄ administration, no net triglyceride release occurred. Treatment of the animal with CCl₄ did not affect the rate of uptake of a palmitate-serum complex or of triglycerides from a synthetic neutral fat emulsion or the production of ketone bodies by the liver. Urea production and glucose release were depressed by CCl₄ poisoning. The synthesis of hepatic triglyceride from acetate-l-C¹⁴ was not accelerated by CCl₄ intoxication. It is suggested that an essential step in the outward transport of hepatic triglyceride is the synthesis of lipoproteins at the endoplasmic reticulum by the utilization of triglycerides previously synthesized at another cellular site. Interference with lipoprotein synthesis by damage to the endoplasmic reticulum, as seen in CCl₄ intoxication, may effectively depress outward triglyceride transport and result in the development of a fatty liver.

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The Action of Carbon Tetrachloride on the Transport and Metabolism of Triglycerides and Fatty Acids by the Isolated Perfused Rat Liver and Its Relationship to the Etiology of Fatty Liver

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