Effect of carbon tetrachloride ingestion on liver and plasma triglyceride turnover rates

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SUMMARY   Specific and total radioactivity of liver and serum triglycerides were determined at various times after palmitate-1-C^{14} injection in carbon tetrachloride-treated and control rats. A multicompartmental model of plasma and liver triglyceride metabolism was used to calculate, with the aid of a digital computer, transfer rates from one compartment to another.

Analysis of the data indicates that carbon tetrachloride ingestion results in a lowering of the rate of hepatic triglyceride secretion to one-tenth of the control value. In addition, the rates of plasma triglyceride turnover and of hepatic uptake of plasma triglyceride were found to be only one-fifth of the control rates. The results are compatible with a hypothesis of disruption of hepatic mechanisms involved in the synthesis of triglyceride–protein complexes, in their secretion, or both.

T HE MECHANISM of hepatic lipid accumulation following carbon tetrachloride ingestion has been studied in many laboratories (1). Recently, the rapid increase in hepatic triglyceride (TG) was shown to account for the entire accumulation of hepatic lipid shortly after carbon tetrachloride ingestion (2, 3). In addition, soon after ingestion of this hepatotoxin by rats it has been observed that (a) plasma TG decreases (4, 5); (b) the striking elevation of plasma TG noted after Triton injection in control animals is absent (4); (c) perfused livers are unable to release net TG into the perfusate (6); (d) incorporation of injected amino acids into plasma lipoproteins is decreased (7); and (e) incorporation of injected palmitate-1-C^{14} into plasma TG is depressed (5).

These observations and the fact that the liver is the chief source of plasma TG in the postabsorptive state (8–10) support the hypothesis (4) that carbon tetrachloride ingestion causes rapid liver TG accumulation by impairing the hepatic TG secretory mechanism.

Recently, we presented an analysis of TG metabolism in normal fed rats (11). In this study a multicompartmental model was constructed and TG flow rates and compartment sizes were calculated from data on the incorporation of palmitate-1-C^{14} into liver and plasma TG. In the present study we have used this model to study the effect(s) of carbon tetrachloride ingestion on triglyceride metabolism in rats.

METHODS

Male Sprague-Dawley rats (135–180 g), previously fasted 20 hr, were given mineral oil (0.25 ml per 100 g body wt) or a CCl$_4$–mineral oil mixture 1:1 (0.5 ml per 100 g body wt) by stomach tube. Four hr later, the rats were lightly anesthetized with ethyl ether and 4.6 μc of palmitate-1-C^{14} (8.87 mc/mmole, Volk Radiochemical Co., Chicago, Ill.), as an albumin complex (12), were injected into the tail vein. At varying times after the palmitate injection, the rats were again anesthetized with ethyl ether, the abdominal aorta was severed, and serum and liver samples were obtained. Chloroform–methanol (2:1) lipid extracts of 1 ml of serum and about 1 g of liver were prepared (13, 14), water washed, dried, and redissolved in chloroform. The TG from an aliquot of the liver and the total serum lipid extracts were isolated by thin-layer chromatography (11). Following quantitative elution from the silicic acid plates (11) aliquots were assayed for TG (15), and C^{14} was determined in a liquid scintillation counter.

The total serum TG per 100 g body wt was obtained by multiplying the mean serum TG concentration (mg/ml) for all rats in a group by the serum volume per 100 g body wt, which was assumed to be 4 ml (16). This product, the total serum TG per 100 g body wt multiplied by the average serum TG specific activity, yields the total
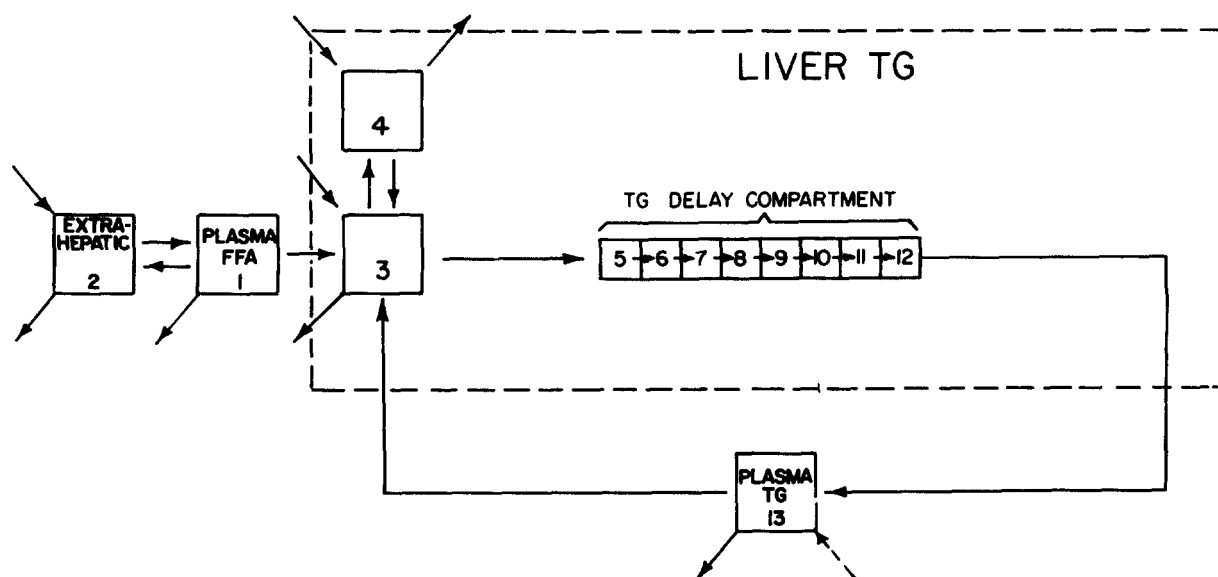
FIG. 1.   A multicompartmental model of TG metabolism.   Liver TG is represented by the compartments enclosed within the broken lines.

serum radioactivity (per cent of injected dose; not corrected for body weight).

THEORETICAL CONSIDERATIONS

The Model

A model of TG metabolism in the intact animal was presented earlier (11) and is shown here in Fig. 1. Compartment *1* of the model represents plasma free fatty acids (FFA) and is depicted with three exits. One exit represents the conversion of plasma FFA to lipids (in hepatic and extrahepatic tissues) which return essentially no FFA into the plasma. The second exit from compartment *1* enters compartment *2* and represents a pathway by which FFA leaves the circulation to enter extrahepatic tissues as free and esterified fatty acids. Fatty acids formed in compartment *2* may also enter or reenter the plasma FFA compartment. The third exit shown represents the flux from plasma FFA into hepatic TG. The latter is depicted as having three major subdivisions, compartments *3*, *4*, and a delay compartment (consisting of subcompartments *5* through *12*). The rationale for dividing hepatic TG in the present "minimal model" of TG metabolism is based on three experimental observations made after injection of labeled FFA into rats (9, 11, 17); (*a*) a portion of the hepatic TG becomes labeled quickly, (*b*) another portion depicted as compartment *4* becomes labeled slowly, (*c*) labeled TG begins to appear in plasma only after a delay of about 10 min. It was found empirically that a series of 8 rapidly turning over compartments would simulate this delay. Plasma TG is represented in this model by compartment *13*.

Two other models have also been considered (reference 11, Fig. 6). One of these considers two possibilities: (*a*) plasma TG may enter a different hepatic TG compartment from that formed from plasma FFA; (*b*) hepatic TG which forms directly from plasma TG may be secreted again into plasma without passing through the delay compartments. The second model considers that the hepatic TG compartment which becomes labeled most slowly does so by incorporating TG fatty acid from the last of the series of delay compartments, which is the same compartment as that which serves as the immediate precursor of plasma TG. Detailed results of analyses using these models will not be presented. Additional experiments are required to establish which of the several possibilities is most nearly correct. However, most of the conclusions emphasized in the present paper under "Multicompartmental Analysis of Data" have been found to be independent of the choice of model (11).

Mathematical Solution

The mathematical solution of rates and pool sizes was accomplished by the computer program of Berman, Weiss, and Shahn (18). Practically, this required our presenting a 7090 computer with initial estimates for each of the fractional flow rate constants in our model, along with experimentally obtained values of liver TG total activity at 7 time points and of serum TG total activity at the same time points. The computer then calculated, using the estimated rate constants and the initial conditions (100% of the radioactivity in compartment *1* at zero time), the activity in liver and plasma at each of the 7 experimental time points. Then by iterative procedures it modified the initial estimates for the

rate constants until a least squares best fit was approached.

The rate constants of plasma FFA leaving plasma and entering compartment 2, and of plasma FFA entering undesignated compartments were not varied during the analysis; thus the plasma FFA compartment in the analysis served as a function generator which supplied hepatic TG with C^{14} at a rate which would result in hepatic TG having the observed total activity–time curve.

Flux of FFA and of TG (mg FFA or TG per min, standardized to 100 g body wt) were calculated from steady state equations and from the measured compartment sizes of liver and serum TG and their fractional rate constants of turnover. In the case of CCl_4-treated rats, hepatic TG was in a nonsteady state during the experiment. Additional assumptions, which are indicated below, were required in order to calculate TG flux under these conditions.

Assumptions

In an earlier paper (11) we noted that there are two types of assumptions which may be required in using the program of Berman et al. (18) to obtain a solution to the present model. One type of assumption is used to formulate initial estimates of rate constants which may subsequently be modified. Another type involves the assumption of values which are not permitted to change during the analysis. The latter are listed below. Although values based on these assumptions will be included in the final "solution" of the model, they are, in fact, not determined by the computer analysis. In the control group, all compartments were assumed to be in a steady state during the experiment. Using published values for rat plasma volume (16) and for plasma FFA concentration (19) we calculated that the plasma FFA compartment was 1 mg per 100 g body wt. A value of 0.7/min for the rate constant for plasma FFA turnover was used.[1]

In CCl_4-treated rats, plasma volume, FFA compartment size, and the rate constant for FFA turnover were assumed to be the same as control values.[1, 2] The last

assumption is supported by the observation that in vitro FFA are released from adipose tissue of CCl_4-treated rats at a normal rate (22). Our experimental data and those of Maling et al. (5) showed that the plasma TG concentration remained virtually constant (steady state) between 4 and 6.5 hr after CCl_4 administration, the time interval during which the isotopic study was performed. However, since hepatic TG continues to accumulate during the experimental period, the hepatic TG compartments were considered to be in a nonsteady state. As a first approximation, and to simplify treatment of the data by the computer program of Berman et al. (18) we have assumed that: (*a*) hepatic TG accumulates at a constant rate from the time that CCl_4 is first given until the end of the isotopic experiment; and (*b*) during the period of isotopic study all fractional rate constants remain unchanged even in compartments which may be changing in size.[3]

The number of delay compartments and their turnover rates were arbitrarily adjusted so that they would cause the observed delay in appearance of TG-C^{14} in plasma.

Approximately 10% of the plasma TG, in the rat, may be derived from extrahepatic tissues in the postabsorptive state (8, 9). This relatively small TG influx into the circulation was not taken into consideration in the control group. However, in the CCl_4-treated group, where hepatic TG secretion may be impaired, the contribution made by extrahepatic tissue to plasma TG probably is not small enough to be ignored. Accordingly, we have assumed TG is secreted by extrahepatic tissues of CCl_4-treated rats at 10 ± 5% of the normal hepatic TG secretion rate.

In both control and CCl_4-treated rats we have assumed that one-third of plasma TG which is removed from the circulation is converted to hepatic TG.[4] This assumption does not influence appreciably the final calculations of the rates of hepatic TG secretion, total hepatic TG turnover, or total plasma TG turnover (11).

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[1] In unpublished experiments we have determined the $t_{1/2}$ of palmitate-C^{14} disappearance in 8 rats treated with mineral oil and 7 treated with CCl_4. These animals were treated similarly to those described in the Methods section of this paper. Between 30 sec and 2 min (3 venous samples/rat) no significant difference in the disappearance rate of palmitate-C^{14} was noted between the two groups. Calculation of the rate constant of plasma FFA turnover from the mean values of the two groups during this interval was 0.7/min. A similar value was reported for fasted rats by Olivecrona et al. (20).

[2] Both T. M. Brody and P. Stern, and R. O. Recknagel and A. K. Ghoshal (personal communications) reported that shortly after CCl_4 ingestion there was no significant difference in plasma FFA concentration from the control value. Other investigators (21) reported increased plasma FFA values in CCl_4-treated rats, but these studies were carried out after a much longer time period following CCl_4 ingestion than the experiments cited above.

[3] The computer program of Berman et al. (18) may be used to obtain the flow rate constant in and out of any compartment in the model in the nonsteady state if the rate constants are not variable during the experiment and the data are expressed as total rather than specific activities. In normal rats, evidence has been presented that the fractional rate constant of plasma chylomicron-TG turnover remains unchanged as the compartment size is varied over a wide concentration range (23). However, in the case of hepatic TG in the nonsteady state no data are available to support this assumption.

[4] In normal rats this assumption is based partly on the results of French and Morris (24). (We know of no published data bearing on this point in CCl_4-treated animals.) Experiments in our laboratory (unpublished) have shown that in fasted rats, the fractional turnover of endogenous very low density lipoprotein TG-C^{14} which is directed towards hepatic TG formation is similar to that estimated from the results of French and Morris (24), who injected large loads of chylomicron TG.

TABLE 1 SERUM AND LIVER TRIGLYCERIDE CONCENTRATIONS IN NORMAL AND CCl₄-TREATED RATS

	No. of Rats	Body Wt*	Liver Wt	Liver TG	Serum TG	
		g	g	mg/100 g body wt	mg/100 g body wt	mg/ml
Control	19	155 ± 12	5.3 ± 0.4	19 ± 10	0.96 ± 0.36	0.24 ± 0.090
CCl₄	21	164 ± 8	6.4 ± 0.5	70 ± 32	0.32 ± 0.10	0.080 ± 0.025

* All values reported as mean ± standard deviation.

RESULTS

Liver and Serum TG Data

CCl₄ ingestion slightly increased the rats' liver weight, markedly elevated the liver TG and lowered the serum TG concentration compared to the control values (Table 1).

The control liver TG specific activity attained a maximum value 10 min after palmitate-1-C¹⁴ injection (Fig. 2). As noted previously (11) the serum TG was essentially unlabeled during the first 10-min period, but its specific activity rose steeply during the next 10 min, and reached a maximum value at 30 min. Serum TG specific activity then fell as an exponential function of time and remained higher than that of liver, with the exception of the 90-min value. The maximum serum TG specific activity was approximately double the highest liver value in the control rats.

The liver specific activity of the CCl₄-treated group rose rapidly and then remained essentially constant (Fig. 2). Its highest value was about half the maximum control specific activity; however, the total radioactivity incorporated into hepatic TG at early times was higher in the CCl₄-treated rats. The serum TG specific activity was virtually zero at 10 min and did not show the rapid rise observed in the control group during the next 10

min. The maximum serum TG specific activity was reached at 40 min and then the values fell as a complex exponential function of time. The maximum serum TG value of the CCl₄ group was approximately one-fifth the maximum control group value (Fig. 2). In the CCl₄-treated group, when the serum reached its maximum specific activity the liver value was almost equal to it. Although this observation is in agreement with a precursor–product relationship the remainder of the curve is inconsistent with this type relationship (25).

MULTICOMPARTMENTAL ANALYSIS OF DATA

The FFA and TG compartment sizes and rates of metabolism and transfer in and between various hepatic and plasma compartments are shown in Fig. 3A and 3B for control and CCl₄-treated rats, respectively. The most noteworthy points that may be emphasized in comparing the two figures are:

1. The hepatic TG secretion rate into plasma in CCl₄-treated rats was only 10% of normal (0.015 compared with 0.15 mg/min per 100 g body wt).

2. The over-all rate of hepatic TG metabolism (transformation to non-TG compounds by hydrolysis, oxidation, etc.) was not markedly affected by CCl₄ treatment. The rates of hepatic TG metabolism are indicated in Figs.
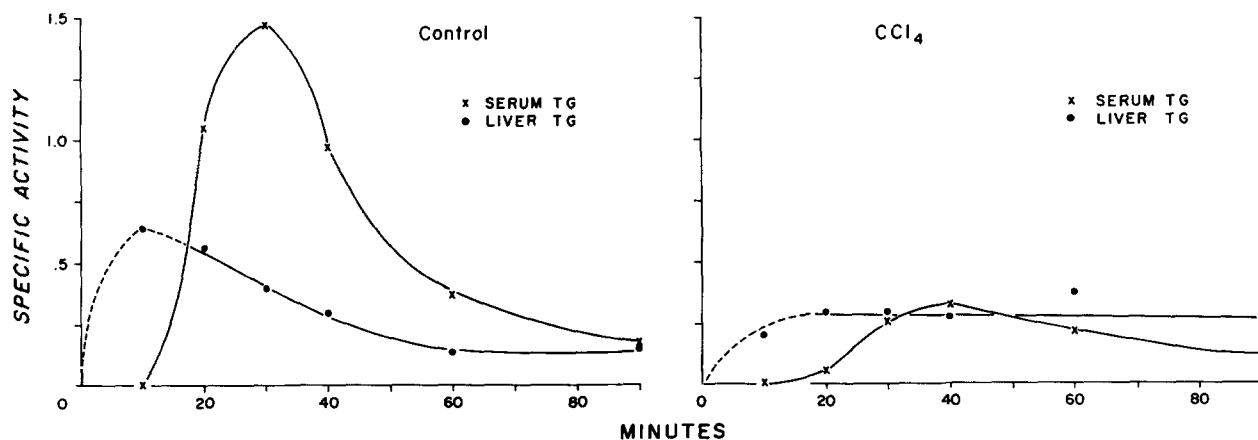

FIG. 2. Liver (●) and serum (×) TG specific activities of control and CCl₄-treated rats. Each point represents the mean value obtained from three rats. Specific activity units are defined in the text. See Fig. 4 for variance. The dotted portion of the liver TG curve during the first 20 min indicates that the initial rise is not well defined.

3A and 3B by arrows leaving the total liver TG compartment (excluding the one entering the plasma TG compartment).

3. The rate at which plasma FFA was esterified to hepatic TG was 46% greater in the CCl₄-treated rats compared to the normals (0.13 as opposed to 0.092 mg/min per 100 g body wt). An even higher rate in the case of CCl₄-treated rats would be expected if the plasma FFA concentration were greater than normal.[2]

4. The portion of total hepatic TG formation that results directly from plasma FFA conversion to TG in liver was about 30% in the controls and 40% in the CCl₄-treated rats. In the normal (see Fig. 3A), for example, this fraction is

$$\frac{0.092}{0.092 + 0.049 + 0.13 + 0.052} = 0.28.$$

(The latter calculation is dependent upon assumed values for plasma FFA concentration and turnover rate).

5. The rates of hepatic TG uptake from plasma TG were 0.052 and 0.011 mg/min per 100 g body weight in
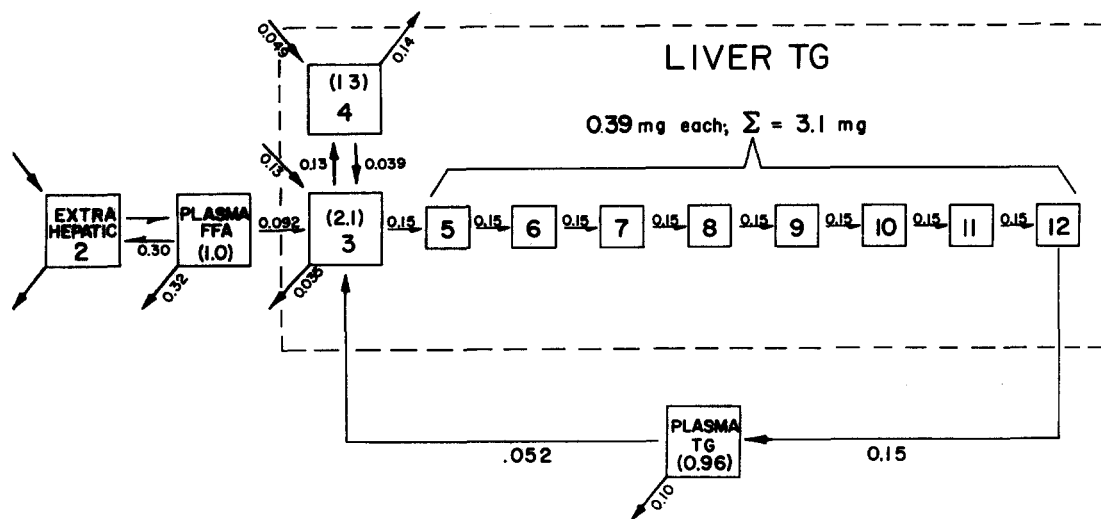

FIG. 3A. Multicompartmental model of TG metabolism in control rats. Liver TG is represented by the compartments enclosed within the broken lines. Compartment sizes are expressed as mg TG or FFA per 100 g body wt; except for "delay compartments" 5–12, compartment sizes are in parentheses. The FFA compartment size was assumed (see text) and those for the total liver and serum TG were obtained from Table 1. No compartment size is assigned to compartment 2. The plasma FFA is designated compartment 1 and the plasma TG compartment 13. Rates indicated by the arrows are expressed in mg TG or FFA per min per 100 g body weight. These values (and those shown in Fig. 3B) when used with the Berman et al. (18) program are the basis of the calculated curves shown in Fig. 4.
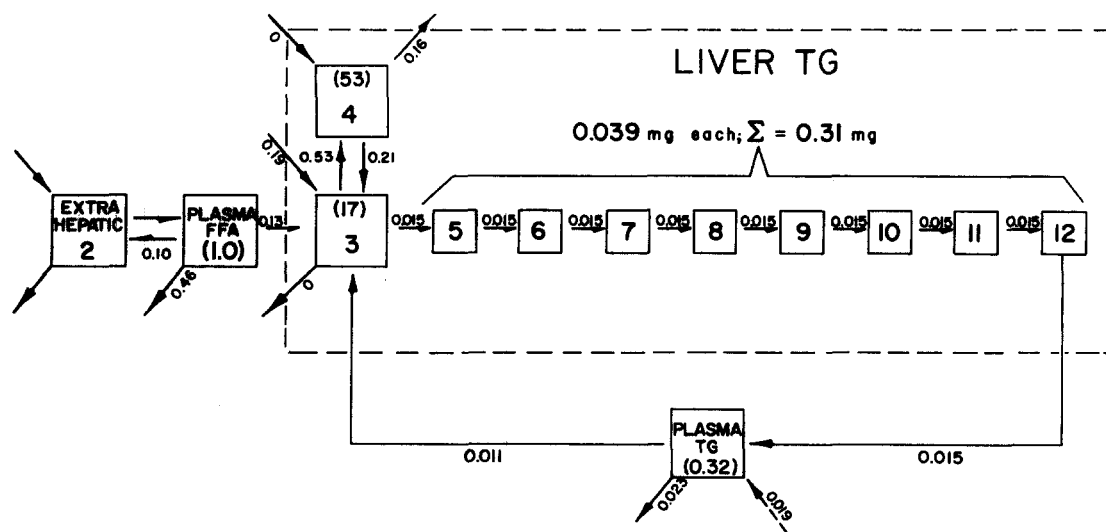

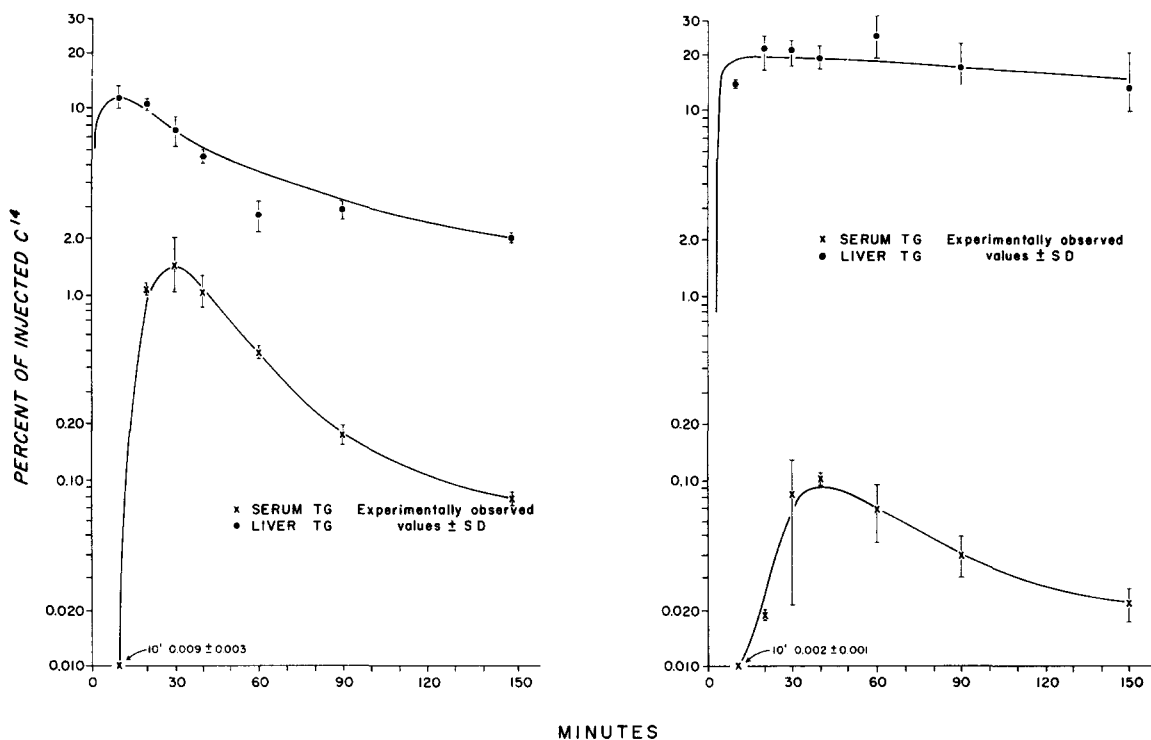FIG. 3B. Model (and solution) of TG metabolism in CCl₄-treated rats.

Fig. 4. Calculated vs. observed serum and liver TG-C^{14} as a time function following injection of palmitate-1-C^{14} in control (left) and CCl_4-treated (right) rats. The ordinate represents the per cent of the injected palmitate-1-C^{14} found in the TG fraction. The curves (solid lines) were computed from the program of Berman et al. (18) using the parameters shown in Figs. 3A and B.

normal and CCl_4-treated rats, respectively. Normally 16% of newly formed hepatic TG was found to be derived directly from plasma TG. In the CCl_4-treated rats this inflow was reduced by four-fifths to a value of 3.3%.

6. The plasma TG uptake by extrahepatic tissues was about one-fourth the normal rate following CCl_4 treatment (normal, 0.10; CCl_4-treated, 0.023 mg/min per 100 g body wt).

7. As noted in Table 1 the plasma TG pool size is decreased to one-third the normal value while hepatic TG was about 4 times the normal value after CCl_4-treatment. The mean rate of hepatic TG accumulation was 0.16 mg/min during the 315 min which elapsed between the time of CCl_4 administration and the midpoint of the C^{14} phase of the experiment. Initially calculations were carried out in which we assumed that hepatic TG which accumulated following CCl_4 treatment were distributed in all hepatic compartments so that each compartment contained the same fraction of the total TG as in the control animals. This assumption was, however, incompatible with other restrictions which had been placed on the model. Similarly, the assumption that only one liver compartment (either 3 or 4) accumulated TG was incompatible with the observed data. We were able to satisfy the condition of hepatic TG accumulation at a mean rate of 0.16 mg/min per 100 g

body wt and also satisfy other limiting relationships by assuming that part of the hepatic TG had accumulated in compartment 3 after CCl_4 ingestion, but prior to palmitate-C^{14} injection. Compartment 3 reached the abnormal size (8 × normal) shown in Fig. 3B, but then remained in a steady state (as did plasma TG) during the period of isotopic study. Compartment 4, which contains TG that is labeled at a slow rate after injection of palmitate-C^{14}, was considered to be the only compartment accumulating TG during the C^{14} phase of the experiment. It contained about four times the normal TG concentration at the midpoint of the period of C^{14} study and was enlarging at the rate of 0.16 mg/min per 100 g body wt.

8. More than half of the plasma TG was derived from extrahepatic sources in CCl_4-treated rats. The restrictions noted in point 7 set this value at 0.019 mg/min per 100 g body wt, which is 12% of the normal hepatic TG secretion rate into plasma. The data from Triton-treated rats (8) indicate that about 86% of the plasma TG is derived from the liver and 14% from extrahepatic sources in normal animals.

Two other models (reference 11, Figs. 6A and B) were also used to compare rates of TG metabolism and flow in and between plasma and liver. The major observations listed above were not altered. As noted previously (11)

rates of hepatic TG turnover, secretion, and formation from plasma FFA as well as the rate of plasma TG turnover are relatively insensitive to the choice of model.

The models' parameters shown in Figs. 3A and B were used with the program of Berman et al. (18) to calculate curves of total radioactivity in plasma and liver as a time function in the two groups of animals. These curves correspond within experimental limits to the observed values as shown in Fig. 4 except for the 60-min liver TG values in both groups of rats.

The data presented also permit an estimation of the maximum contribution that hepatic TG oxidation might make to CO_2 formation in the control rats. Baker et al. (26) found that the overnight-fasted rat (same sex, weight, and strain as in present study) excreted CO_2 at a rate of 0.084 mmoles/min per 100 g body wt, about 10% of which was derived from the oxidation of glucose. Therefore, 0.076 mmole of CO_2 per min per 100 g body wt was derived from combustion of non-glucose compounds. The maximum contribution which TG oxidation in the liver might make to the over-all oxidation rate of non-glucose compounds may be set at 0.012 mmole of CO_2 per min per 100 g body wt (assuming that 0.14 + 0.035 mg TG per min were oxidized completely to CO_2 (see Fig. 3A)). If all the TG secreted by the liver into plasma were eventually metabolized completely to CO_2 an additional 0.010 mmole of CO_2 per min per 100 g body wt would be expected to form. The sum of these figures represents 26% of the total CO_2 production and is the maximum amount of CO_2 which might be derived from TG formed in the liver.

The present analysis, though complicated, has been carried out using a highly oversimplified model. We anticipate that calculations of rates and pool sizes will be subsequently revised as more sophisticated models are developed from subsequent biochemical and kinetic studies. The formulation of the present model (11) was intended to serve as a basis for comparing relative rates of TG flux under various physiological conditions and to stimulate further experimentation which should test the model's validity.

DISCUSSION

The rate of hepatic TG secretion into plasma in rats fasted overnight was 0.15 mg/min per 100 g body wt. An even more rapid hepatic TG secretion rate was reported earlier in glucose-fed rats (11). Laurell (27) also reported that endogenous plasma TG are replaced at rates consistent with our data. We have calculated that the hepatic TG secretory rate in rats is sufficiently fast to account for the rapid changes in plasma and liver TG concentrations after various treatments considered to block TG exit from plasma (8) or liver (4).

A major aim of this study was to determine, employing isotopic tracer techniques, whether CCl_4 ingestion impairs hepatic TG secretion in the rat. Previous attempts to measure the effect of this hepatotoxin on hepatic TG secretion utilized three different methods: blocking of plasma TG exit with a detergent (Triton) followed by measurement of plasma TG (4); determination of net TG output from perfused livers (6); and incorporation of palmitic acid-C^{14} in vivo into liver and plasma TG (5). Interpretation of all these data is difficult. Triton may have other effects in addition to inhibition of plasma TG egress (8). In the perfusion study, measurements of net TG changes in the media were not necessarily a true indication of the maximal secretory rate into plasma. In the C^{14} studies, although differences in palmitate-C^{14} incorporation into plasma and liver TG were reported, no attempt was made to calculate the rate of hepatic TG secretion. The data presented in this paper indicate that the hepatic TG secretory rate in CCl_4-treated animals is one-tenth the normal rate. An even more drastic reduction of the hepatic TG secretory rate might be detected if measurements were made on the different lipoprotein fractions constituting the plasma TG compartment. While the present method also suffers from uncertainties, none of these would alter the conclusion that CCl_4 causes a drastic reduction in the rate of hepatic TG secretion.

In addition to the pronounced effect on the hepatic TG secretory rate CCl_4 ingestion was found to markedly reduce hepatic uptake of plasma TG. Heimberg et al. (6) have studied uptake of plasma TG by perfused liver, in vitro, from rats which had received CCl_4 dissolved in mineral oil. We have interpreted their data to indicate that ingestion of CCl_4-mineral oil decreases hepatic uptake of plasma TG compared to the mineral oil-fed controls.[5] This difference seems significant to us since both the control and experimental livers were perfused with the same TG concentration in the media. In vivo, however, livers of CCl_4-treated rats are bathed in plasma containing only one-third as much TG as controls (4, 5). Since Belfrage et al. (23) have shown that hepatic TG uptake is proportional to the plasma TG concentration in normal rats, a more marked decrease in uptake would have been expected in the perfused liver from the CCl_4-treated rats had the medium TG concentration been reduced by at least two-thirds.

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[5] Heimberg et al. (6) compared the groups of rats receiving CCl_4 plus mineral oil with both a mineral oil-treated and non-treated group. They suggested that uptake of plasma TG by liver is normal in perfused liver of CCl_4-treated rats. This interpretation is based on comparison of the non-treated group with the CCl_4-mineral oil treated group. It is possible, however, to reach a different conclusion by comparing the CCl_4-mineral oil group with the mineral oil group.

In the fasted rat, the liver is virtually the sole source of plasma TG (8, 10) and yet this organ extracts TG from the plasma faster than any other tissue (23). These observations have led us to formulate the following working hypothesis. Proteins, like albumin, that are secreted by the liver into the circulation may form intracellular complexes with TG that enable the protein to pass through the lipoprotein cell membrane. Using albumin as an example, this hypothesis requires that TG dissociate from the protein complex as it leaves the liver cell (at the membrane or in blood); and that TG then form a typical plasma lipoprotein and/or return as TG to form complexes with more intracellular hepatic protein. Studies of experimental nephrosis in which protein secretion by liver is increased show augmented hepatic TG uptake and secretion (28). Conversely, our results show decreased hepatic uptake and secretion in CCl_4-treated rats, the livers of which synthesize plasma protein at an abnormally slow rate (7, 29). All these findings are compatible with this hypothesis. It will be interesting to see whether other inhibitors of hepatic protein synthesis and secretion inhibit both hepatic TG uptake and secretion. It is of interest that newly synthesized albumin is found in the rat liver microsome fraction bound to lipids (30).

Although our attention has been focused upon liver lipids following CCl_4 ingestion, analysis of the data indicates that CCl_4-treatment also strongly inhibits TG uptake by extrahepatic tissue. The reduced uptake of plasma TG by hepatic and extrahepatic tissues may merely reflect the low concentration of very low density lipoprotein TG in the CCl_4-treated rats rather than a defect in the mechanism of TG penetration into cells. We are not aware of any studies on the effect of lowering the concentration of endogenously synthesized very low density lipoprotein TG on hepatic and extrahepatic TG uptake. However, Belfrage et al. (23) have shown that the disappearance rate of injected chylomicron TG from plasma is a function of the circulating TG concentration. The present analysis assumes without an experimental basis that about one-third of the total plasma TG which turns over is converted to liver TG in the CCl_4-treated rat. French and Morris (31) found in rats that approximately one-third of administered chylomicron TG which turned over was incorporated into liver lipids. However, the observation that the total plasma TG turnover rate is greatly reduced in CCl_4-treated rats is independent of this assumption.

The rate of FFA conversion to hepatic TG was estimated to be higher in CCl_4-treated than in control rats. The significance of this difference is questionable in the light of the several assumptions which were made in calculating this rate. Maling et al. (5) have presented evidence that would indicate that the rate of palmitate-1-

C^{14} incorporation into liver TG is 10 times faster in CCl_4-treated rats than in controls. However, the data shown in their Figs. 4 and 5 for control animals in which liver TG total activity and specific activity are shown to rise rapidly only after a 20-min delay are in complete disagreement with the present data as well as with all similar experiments that we know of in various species of animals (9–11, 17, 19, 27, 32).

Havel (33) and Friedberg et al. (34) have estimated the fraction of plasma TG which is derived from plasma FFA in overnight-fasted humans. In each study, the authors concluded that all of the plasma TG is probably derived from plasma FFA. Friedberg et al.'s (and, presumably, Havel's) method of calculation is based upon a simple model which consists of a plasma FFA compartment and a combined liver and plasma TG compartment. The assumption that liver and plasma TG constituted a single pool was necessary in the absence of any liver data. However, as noted earlier (11) and as shown in the present study, a 2-pool model cannot be used to describe TG metabolism in rats. In the present investigation, we have estimated that, in both fasted CCl_4-treated and control rats, only about 35% of the newly formed hepatic TG was derived directly from plasma FFA. A similar conclusion was reached in our study of carbohydrate-fed rats (11). Since lipogenesis from glucose is inhibited in fasted rats (35), we would hypothesize that the relatively weakly labeled source of hepatic (and hence also of plasma) TG may be lipid such as phospholipid, which could be derived, in part, from plasma FFA.

The estimated rates of plasma FFA conversion to hepatic TG have not been determined with accuracy in the present study. To measure the rate of hepatic TG formation from plasma FFA a detailed analysis of plasma and tissue FFA and of individual hepatic lipid specific activities and pool sizes is required during the first few minutes after injection of labeled fatty acid. Further experiments may be expected to modify the present model of TG metabolism in rats. As more sophisticated models are evolved we would anticipate that better estimates of the above parameters may be obtained. However, the results of the present study are clearly compatible with the hypothesis that rapid liver TG accumulation following CCl_4 ingestion is due to disruption of the hepatic mechanism(s) involved in the synthesis of TG–protein complexes, in their secretion, or in both (4–7, 36).

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