INTERFERENCE WITH SECRETION OF TRIGLYCERIDES BY THE LIVER AS A COMMON FACTOR IN TOXIC LIVER INJURY

With Some Observations on Choline Deficiency Fatty Liver

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Fatty infiltration of the liver may be induced in experimental animals by a wide variety of toxic substances, metabolites or nutritional deficiencies. Despite the heterogeneity of these etiologic factors, the lipids accumulating in the liver appear to be predominantly triglycerides in nature.[1-7] This fact suggests the likely possibility that some key cellular derangement, intimately linked to hepatic triglyceride metabolism, is common to all these various conditions. With regard to carbon tetrachloride poisoning, it has been possible, on the basis of work from this laboratory [8] to formulate a simple hypothesis regarding the rapid increase in liver triglycerides [1] which occurs following oral administration of this hepatotoxin. The hypothesis takes into account recent findings in the field of lipid mobilization and transport, made in a number of laboratories, and may be formulated as follows: The liver is constantly secreting large quantities of triglycerides into the plasma. The source of the fatty acids for these plasma triglycerides is plasma nonesterified fatty acids (NEFA), which come from the adipose tissue. Carbon tetrachloride poisons the liver in such a way that entrance of plasma NEFA into the liver and esterification of NEFA to triglycerides is relatively unaffected, whereas exit of liver triglycerides into the plasma is inhibited. As a result, triglycerides accumulate in the liver. Data in support of this hypothesis were obtained in experiments with a simple design.[8] The ability of the liver to secrete triglycerides into the plasma was tested in rats by the intravenous injection of Triton (WR-1339, Winthrop Laboratories). Intravenous administration of this nonionic detergent produces a marked hyperlipemia due initially to an increase in plasma triglycerides.[9] Triton blocks the exit of triglycerides from the plasma com-

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partment[9,10] so that triglycerides, secreted by the liver,[11] accumulate in the plasma. Failure of a post-Triton hypertriglyceridemia to appear in carbon tetrachloride-poisoned rats was taken as evidence of an impaired secretion of liver triglycerides into the plasma.[8]

The experimental design of the carbon tetrachloride-Triton experiments offered a ready opportunity to determine whether interference with triglyceride secretion by the liver could be held responsible for hepatic fat accumulation caused by other hepatotoxins and by nutritional deficiency states. Results reported in this paper show that in rats with elevated liver triglycerides due to prior administration of ethionine, phosphorus, or cerium, the hepatic triglyceride secretory mechanism was inhibited or partially destroyed. Essentially negative results obtained with dietary choline deficiency studies indicated either that the hypothesis in its present form was oversimplified or that it did not apply to choline deficiency fatty liver. A preliminary report of this work has appeared.[12]

Experimental Methods

Experimental Design

Albino rats of the Sprague-Dawley strain were used. Details regarding sex and body weight are given in the tables. Except when noted, the rats were fasted for 16 hours before treatment and throughout the subsequent experimental period. Water was allowed *ad libitum*. In each experiment there were 4 groups of animals: (a) control rats; (b) rats with induced fatty liver; (c) control rats treated with Triton; and (d) rats with induced fatty liver and treated with Triton. A maximum of 8 rats, 2 per group, were placed under experimentation at one time. Treatment and sacrifice of the animals were spaced at intervals of 5 minutes. To increase the number of animals per group, the experiment was repeated on successive days.

Hepatotoxins

DL-ethionine or white phosphorus was administered by stomach tube under light ether anesthesia. DL-ethionine was given at zero time and 2 hours thereafter in 2 equal doses, each of 500 mg. per kg. of body weight. A 2.5 per cent (w/v) solution in 0.9 per cent NaCl solution was used. White phosphorus was administered in a single dose of 7.5 mg. per kg. of body weight. A 0.5 per cent (w/v) solution in mineral oil was used. Control rats received an equivalent amount of either 0.9 per cent NaCl or mineral oil, respectively. Cerium was injected into a saphenous vein under light ether anesthesia, in a single dose of 5 mg. per kg. of body weight. A 1 per cent solution, prepared according to Snyder, Cress and Kyker,[3] was used. Control rats received saline.

Choline-Deficient Diet

Choline-deficient and choline-supplemented purified diets were prepared essentially according to Young, Lucas, Patterson and Best, basal B diet.[13] The choline-supplemented diet contained 0.3 per cent choline. Details of feeding schedules are given in the tables.

Blood and Liver Samples

Triton was injected into a saphenous vein under light ether anesthesia, 90 minutes before sacrifice of the rats. The dose was 100 mg. of Triton per rat, except for the experiments with choline-deficient diets, where the dose was 50 mg. per rat. A 20

per cent solution (w/v) in 0.9 per cent NaCl was used. Control rats not receiving Triton were injected with 0.5 ml. of saline. Ten minutes before sacrifice, the rats were anesthetized with sodium pentobarbital, 5 mg. per 100 gm. of body weight, intraperitoneally. After 10 minutes, 5 ml. of blood were withdrawn from the abdominal aorta and transferred to tubes containing 0.5 ml. of ice-cold 3.8 per cent (w/v) sodium citrate solution. The liver was removed and weighed, and a weighed sample from the left lobe was stored at −10° for lipid analysis. When all of the rats treated at one time had been sacrificed, plasma samples were prepared by centrifuging the blood for 20 minutes at 600 × *g* at 4°. Lipid analyses on plasma and liver were begun immediately.

Analytic Procedures

Total lipids were extracted from samples of liver (1.2 to 1.5 gm.), essentially according to Folch, Lees and Sloane Stanley,[14] dried down under a stream of air at room temperature, then redissolved in chloroform. Aliquots of the total lipid extracted in chloroform were applied to silicic acid columns. The neutral lipids were eluted with chloroform and triglycerides estimated according to van Handel and Zilversmit.[15,16] Neutral lipids of 1 ml. samples of plasma were extracted with chloroform and zeolite. The extract was filtered and triglycerides estimated on aliquots of the filtrate.[15,16]

Results

Ethionine Fatty Liver

Groups of female rats were sacrificed 4, 8, and 24 hours after the first administration of ethionine (Table I). After 4 hours there was a small but statistically insignificant increase in liver triglycerides. After 8 and 24 hours liver triglyceride levels had increased to 3.5 and 7.7 times the respective control levels. After 4 hours the level of plasma triglycerides was only ⅓ that of the controls, and remained low thereafter. It appeared from these results that following ethionine administration, accumulation of triglycerides in the liver was accompanied by, if not preceded by, a drop in the plasma triglyceride level. Following Triton administration the expected rise in plasma triglycerides was observed in control rats, whereas in ethionine-poisoned rats the post-Triton hypertriglyceridemia was much reduced. Depression of post-Triton hypertriglyceridemia was particularly marked 24 hours after ethionine feeding. Injection of Triton had no effect on hepatic triglyceride concentration in control rats nor in ethionine-fed rats. The early appearance of depressed plasma triglycerides in ethionine poisoned rats, and the depression of the ability of these rats to produce a triglyceridemia following Triton administration, indicated that interference with the hepatic mechanism which transfers triglycerides to the plasma was an early feature in the development of ethionine-induced fatty liver.

Since Farber, Koch-Weser and Popper[17] have shown that male rats do not develop a fatty liver following ethionine administration, it appeared interesting to determine whether a sex difference existed also

TABLE I

PLASMA AND LIVER TRIGLYCERIDE CONTENT IN TRITON TREATED AND NON-TRITON TREATED FEMALE RATS AFTER ETHIONINE ADMINISTRATION

Hr. after ethionine	Triglycerides of	No. of rats	Control (no Triton) (mg.)*	No. of rats	Ethionine fed (no Triton) (mg.)	No. of rats	Control (plus Triton) (mg.)	No. of rats	Ethionine fed (plus Triton) (mg.)
4	Liver	4	54.7 ± 12.3	4	71.2 ± 5.0	4	54.5 ± 9.4	4	65.73 ± 6.3
	Plasma	4	17.8 ± 2.0	4	5.7 ± 1.1	4	391.8 ± 30.2	4	251.6 ± 19.6
8	Liver	13	36.1 ± 4.2	14	125.5 ± 9.6	14	31.5 ± 3.1	14	111.8 ± 10.0
	Plasma	12	15.2 ± 1.3	14	9.5 ± 1.5	14	311.2 ± 25.6	14	144.6 ± 15.5
24	Liver	12	33.7 ± 5.5	12	260.0 ± 28.0	12	32.4 ± 4.0	12	246.5 ± 25.1
	Plasma	12	21.4 ± 1.6	12	8.8 ± 1.2	11	305.6 ± 17.3	12	99.8 ± 9.5

Female rats (Holtzman) 134 to 239 gm. body weight. Triton or 0.9 per cent NaCl was administered 90 minutes before sacrifice.

* Liver triglyceride (TG) given as mg. per liver per 100 gm. of body weight, mean ± standard error; plasma TG given as mg. per 100 ml. of plasma, mean ± standard error.

with regard to the action of ethionine on the mechanism which transfers liver triglycerides to the plasma. In confirmation of Farber and coworkers, no significant increase in liver triglycerides was observed in male rats 24 hours after ethionine administration (Table II). However,

TABLE II

PLASMA AND LIVER TRIGLYCERIDE CONTENT IN TRITON TREATED AND NON-TRITON TREATED MALE RATS 24 HOURS AFTER ETHIONINE ADMINISTRATION

Treatment of animals	No. of rats	Plasma triglycerides (mg. per 100 ml. of plasma)	Liver triglycerides (mg.)*
Controls	5(6) †	35.9 ± 2.72	18.1 ± 1.57
Ethionine fed	6	15.2 ± 0.73	23.4 ± 2.09
Triton (no ethionine)	5(6)	260.6 ± 10.10	19.8 ± 2.79
Ethionine fed plus Triton	6	134.3 ± 8.47	21.5 ± 1.63

Male rats (Holtzman) 202 to 288 gm. body weight, sacrificed 90 minutes after intravenous administration of either Triton or 0.9 per cent NaCl.

* All data given as mean ± standard error. Liver TG given as mg. per liver per 100 gm. of body weight.

† Plasma TG, 5 rats; liver TG, 6 rats.

the level of plasma triglycerides and the post-Triton hypertriglyceridemia were depressed in the intoxicated rats to almost the same extent as in female rats given ethionine. From these data it would appear that the sex difference in hepatic triglyceride accumulation was based on factors other than the sensitivity of the hepatic triglyceride secretory mechanism to ethionine.

Phosphorus Fatty Liver

Groups of male rats were sacrificed 8 and 24 hours after administration of phosphorus (Table III). After 8 hours there was a small but statistically insignificant increase in hepatic triglyceride content. The level of plasma triglycerides did not change significantly at 8 hours, nor was the post-Triton triglyceridemia depressed at this time. After 24 hours, liver triglycerides were elevated 5-fold, plasma triglycerides were reduced to ⅓ of control levels, and post-Triton triglyceridemia was even more depressed. As in ethionine feeding experiments, Triton had no effect on hepatic triglyceride concentration in control rats, nor in phosphorus-poisoned rats. It thus appears that 24 hours after phosphorus poisoning, accumulation of triglycerides in the liver was accompanied by a fall in plasma triglycerides and a reduced response to intravenous injection of Triton. In similar experiments no significant changes in either liver triglycerides, plasma triglycerides, or post-Triton hypertriglyceridemia were observed 4 and 12 hours after phosphorus poisoning.

TABLE III

PLASMA AND LIVER TRIGLYCERIDE CONTENT IN TRITON TREATED AND NON-TRITON TREATED MALE RATS AFTER PHOSPHORUS POISONING

Hours	Triglycerides of	No. of rats	Control (no Triton) (mg.)*	No. of rats	Phosphorus (no Triton) (mg.)	No. of rats	Control (plus Triton) (mg.)	No. of rats	Phosphorus (plus Triton) (mg.)
8	Liver	6	16.1 ± 2.5	6	24.0 ± 3.0	6	18.8 ± 2.6	6	26.9 ± 5.8
	Plasma	6	15.0 ± 1.5	6	11.3 ± 1.9	6	241.3 ± 25.1	6	242.4 ± 16.8
24	Liver	8	15.1 ± 1.7	7	74.0 ± 5.3	8	17.9 ± 2.4	7	75.5 ± 12.0
	Plasma	8	23.0 ± 2.5	6	8.1 ± 2.6	8	264.1 ± 13.1	7	63.7 ± 5.5

Male rats (Holtzman) 202 to 274 gm. body weight, sacrificed 90 minutes after intravenous administration of either Triton or 0.9 per cent NaCl.

* Liver TG given as mg. per liver per 100 gm. of body weight; plasma TG given as mg. per 100 ml. of plasma.

Cerium Fatty Liver

Groups of female rats were sacrificed 24 hours after intravenous administration of cerium. Twenty-four hours after administration of the rare earth, liver triglycerides were increased 4-fold (Table IV). Injection of Triton was again without effect on hepatic triglyceride levels.

TABLE IV

PLASMA AND LIVER TRIGLYCERIDE CONTENT IN TRITON TREATED AND NON-TRITON TREATED FEMALE RATS 24 HOURS AFTER CERIUM ADMINISTRATION

Treatment of animals	No. of rats	Plasma triglycerides (mg. per 100 ml. of plasma)	Liver triglycerides (mg.)*
Controls	12(10) †	15.6 ± 0.5	38.2 ± 5.7
Cerium	12(11)	14.5 ± 1.5	132.6 ± 11.5
Triton (no cerium)	12(11)	315.1 ± 21.6	33.1 ± 4.2
Cerium plus Triton	12	108.4 ± 19.3	132.9 ± 13.9

Female rats (Holtzman) 186 to 224 gm. body weight, sacrificed 90 minutes after intravenous injection of either Triton or 0.9 per cent NaCl.

* All data given as mean ± standard error. Liver TG given as mg. per liver per 100 gm. body weight.

† Plasma TG, 12 rats; liver TG, 10 rats.

Contrary to the findings with ethionine and phosphorus, no reduction in plasma triglycerides was observed in animals with fatty livers. Control rats injected with Triton exhibited the expected post-Triton hypertriglyceridemia. The post-Triton hypertriglyceridemia elicited in cerium-poisoned rats was only 30 per cent of that noted in the Tritonized controls. Although no fall in the level of plasma triglycerides was seen in cerium-poisoned, non-Tritonized rats, the data nevertheless suggest the existence of a cerium-induced defect in the hepatic triglyceride secretory mechanism since, when challenged with Triton, the poisoned rats exhibited a markedly reduced hypertriglyceridemia. For the validity of the hypothesis on which these experiments are based, we regard the decreased post-Triton hypertriglyceridemia in cerium-poisoned rats as more important than the failure of non-Tritonized, cerium-poisoned rats to exhibit a fall in plasma triglycerides. On the assumption that Triton blocked the exit of triglycerides from the plasma, the rate of accumulation of triglycerides in the plasma compartment in Tritonized animals was a measure of the rate of secretion of triglycerides by the liver. In non-Tritonized animals, however, the plasma triglyceride level at any time depended on both entrance and exit of triglycerides from the plasma compartment. If the total pathologic situation produced by cerium poisoning inhibited exit of triglycerides from the plasma compartment as well as their exit from the liver, a fall in plasma triglycerides might not

be observed even though the hepatic secretory mechanism was inhibited or partially destroyed.

Choline Deficiency Fatty Liver

After an overnight fast of 17 hours, male rats, weighing 110 to 134 gm., were offered 20 gm. of a choline-deficient diet from 12 noon until 9:30 a.m. of the following day, when either 0.9 per cent NaCl or Triton was injected intravenously. Samples of plasma and liver were taken for triglyceride analysis after 90 minutes. Food intake was recorded and two control groups, fed either a choline-supplemented diet or ground chow (Purina Laboratory Chow) were pair-fed against the choline-deficient group. Liver triglycerides of the choline-deficient rats were elevated 4-fold over chow-fed controls (Table V). Liver triglycerides

TABLE V

PLASMA AND LIVER TRIGLYCERIDE CONTENT IN TRITON TREATED AND NON-TRITON TREATED MALE RATS FED A CHOLINE-DEFICIENT DIET

Diet	Treatment of animals *	Plasma triglycerides (mg. per 100 ml. of plasma)	Liver triglycerides (mg.)†
Chow	No Triton	15.7 ± 2.1	29.4 ± 6.5
Chow	Plus Triton	152.0 ± 30.7	26.9 ± 4.6
Choline supplemented	No Triton	33.6 ± 8.4	39.4 ± 3.6
Choline supplemented	Plus Triton	360.5 ± 39.8	39.9 ± 5.7
Choline deficient	No Triton	18.2 ± 1.2	110.4 ± 11.5
Choline deficient	Plus Triton	247.3 ± 57.9	136.7 ± 6.9

* Either 0.9 per cent NaCl or 50 mg. of Triton in 0.9 per cent NaCl was injected intravenously 90 minutes before sacrifice of the animals.

† All data given as mean ± standard error. There were 4 rats per group. Liver TG given as mg. per liver per 100 gm. of body weight.

of the rats fed a choline-supplemented diet were elevated only 25 per cent over chow-fed controls. Liver triglycerides of Tritonized rats were not significantly different from non-Tritonized controls on the same dietary regimen.

The findings with respect to plasma triglycerides did not fit the expected pattern. The level of plasma triglycerides in choline-deficient rats was reduced to about ½ the level of the group receiving a choline-supplemented diet, but was not significantly different from plasma triglycerides of rats fed ground chow. However, 90 minutes after Triton administration, the choline-deficient rats exhibited a marked hypertriglyceridemia, which was not significantly different from either the Tritonized choline-supplemented controls ($p>0.1$) or the Tritonized chow-fed controls ($p>0.1$). This observation was distinctly contrary to expectation.

The experiment reported in Table V was repeated as follows: Male rats (Holtzman), weighing 78 to 98 gm., were fasted overnight, then offered 20 gm. of a choline-deficient diet at noon. After 24 hours, food was removed, and the rats again fasted overnight until 9 a.m. of the following day, when either 0.9 per cent NaCl or Triton was administered intravenously. Samples of plasma and liver were taken for triglyceride analysis after 90 minutes. Rats pair-fed a choline-supplemented diet, but otherwise treated exactly as above, served as controls. Liver triglycerides of the choline-deficient rats were elevated 8-fold over the rats fed a choline-supplemented diet. However, 90 minutes after Triton administration, plasma triglycerides of choline-deficient rats had increased 19-fold to 353 mg. per cent. In rats fed a choline-supplemented diet, the post-Triton hypertriglyceridemia increased 15-fold to 341 mg. per cent. This experiment confirmed the result shown in Table V—viz., that rats with fatty livers due to choline deficiency did not exhibit a depression in their capacity to develop a post-Triton hypertriglyceridemia.

The possibility was considered that the above experiments did not constitute a sufficiently rigorous test of a possible defect in the ability of choline-deficient rats to produce a post-Triton hypertriglyceridemia. Liver triglycerides increase rather slowly in rats on choline-deficient diets. Conversely, the total flow of fatty acids through the hepatic triglyceride secretory mechanism is large. Consequently, a small depression in the capacity of the liver to secrete plasma triglycerides would result eventually in a significant increase in liver triglycerides, but a small defect in the capacity of the liver to produce plasma triglycerides, as tested with Triton, might be missed if the interval following Triton administration were too short. To test this possibility, the interval following Triton administration was increased to 12 hours (Table VI). Male rats were fasted overnight, then offered either ground chow, the choline-supplemented semi-synthetic diet, or the choline-deficient diet. After 3 days on these diets, all food was withdrawn. The rats were again fasted overnight for 16 hours, after which they received either 0.9 per cent NaCl or Triton intravenously. After 12 hours, plasma and liver samples were taken for triglyceride analysis. The data in Table VI clearly show that rats on a choline-deficient diet, with markedly elevated liver triglyceride levels, did not exhibit any defect in their capacity to produce a post-Triton hypertriglyceridemia.

Discussion

It has been widely appreciated for many years that the pathologic accumulation of fat in the liver of experimental animals and in man could be the result of a malfunction of lipid transport mechanisms. Ear-

lier workers[18] believed that the specific nature of the lesion involved failure of a hepatic mechanism which transported liver fatty acids to plasma in ester linkage as phospholipids. This phospholipid hypothesis was based on the favorable hydrophilic properties of phospholipids in comparison to water-insoluble triglycerides, and especially on the path-

TABLE VI

PLASMA AND LIVER TRIGLYCERIDE CONTENT IN MALE RATS AFTER 3 DAYS ON CHOLINE-DEFICIENT DIET AND 12 HOURS AFTER TRITON ADMINISTRATION

Diet	Treatment of animals *	No. of rats	Plasma triglycerides (mg. per 100 ml. of plasma)	Liver triglycerides (mg.)†
Chow	No Triton	6	22.9 ± 3.9	11.4 ± 2.2
Chow	Plus Triton	6	1026.0 ± 272.0	20.8 ± 4.2
Choline supplemented	No Triton	6	29.7 ± 3.3	32.2 ± 7.0
Choline supplemented	Plus Triton	6	662.0 ± 86.6	24.5 ± 3.8
Choline deficient	No Triton	5	28.2 ± 4.0	355.0 ± 54.5
Choline deficient	Plus Triton	6(5)	1639.0 ± 194.0	245.7 ± 63.6

* Either 0.9 per cent NaCl or 50 mg. of Triton in 0.9 per cent NaCl was injected intravenously 12 hours before sacrifice of the animals.

† All data given as mean ± standard error. Liver TG given as mg. TG per liver per 100 gm. of body weight.

ologic consequences of a choline-deficient diet and the corresponding lipotropism of choline. The phospholipid hypothesis was abandoned when studies with radioactive isotopes conclusively demonstrated[19] that the turnover rate of plasma triglycerides was much more rapid than the turnover rate of plasma phospholipids.

More recently the most cogent hypothesis advanced regarding the specific lesion underlying the pathologic accumulation of liver fat was the mitochondrial hypothesis of Dianzani and Mariani[20] and Christie and Judah.[21] The pioneering work of these investigators opened a new chapter in the study of fatty liver disease since it directed attention to study of enzymic changes in subcellular fractions derived from livers of rats previously treated with hepatotoxins. The main conclusion of the preliminary work—viz., that the specific hepatic lesion involved failure of fat oxidation due to mitochondrial damage—has been shown to be untenable by work from a number of laboratories.[22-26] Although mitochondrial damage became extensive in the period about 14 to 20 hours after carbon tetrachloride poisoning, in the early stages of the disease, when liver triglycerides were increasing rapidly,[1] mitochondrial damage had not occurred.[23] A further weakness of the mitochondrial hypothesis, which postulated depressed levels of liver cell adenosine triphosphate and coenzyme A as contributing to failure of fat oxidation, is the failure to account for the fact that the triglycerides which accumulated in the liver were synthesized from plasma NEFA, implying adequate levels of

these co-factors for triglyceride synthesis. Rather than being mitochondrial, the specific hepatic lesion underlying fat accumulation following toxic liver injury appeared to involve a poorly understood triglyceride secretory mechanism probably associated with the endoplasmic reticulum,[23] and possibly the cell membrane. Although present knowledge does not permit a detailed description of the hepatic triglyceride secretory mechanism, the existence of such a mechanism is well established. In particular, the work of Stein and Shapiro[27] and especially of Laurell[28] showed that a large fraction of C^{14}-labeled nonesterified fatty acids, injected intravenously into rats, entered the liver rapidly and shortly thereafter appeared in the plasma as triglycerides. Byers and Friedman[11] showed that the liverless rat failed to develop a post-Triton hypertriglyceridemia, indicating that the liver is the major source of plasma triglycerides. Borgström and Olivecrona[29] recently showed that when palmitic acid-1-C^{14} was given intravenously to rats in which liver blood flow had been prevented, the circulating plasma triglycerides remained completely free of radioactivity, whereas in animals with liver blood flow intact the circulating plasma triglycerides soon became labeled. Essentially similar results were reported by Havel and Goldfien[30] for hepatectomized dogs. These studies indicate that the liver is the chief site of conversion of plasma NEFA to plasma triglycerides.

Since plasma triglycerides exist as plasma lipoprotein, it can be presumed that the hepatic triglyceride secretory mechanism involves biosynthesis and secretion of plasma lipoproteins. Such a process would require synthesis of triglycerides from plasma NEFA, coupling of these triglycerides with small amounts of phospholipids, cholesterol, cholesterol esters and lipoprotein into the definitive plasma lipoprotein, and eventual secretion of the lipoprotein into the plasma. Failure of such a complex mechanism could be brought about by different agents acting at different levels. Whereas carbon tetrachloride may destroy the entire mechanism outright, with the exception of the initial step leading to formation of triglycerides from plasma NEFA, other steatogenic agents could have a more specific action. Any hepatotoxin or nutritional deficiency which allowed conversion of plasma NEFA to liver triglycerides, but which interfered with the supply of components necessary for formation of plasma lipoprotein, or with the assembly of these components into the definitive lipoprotein molecule, or with its secretion, would result in pathologic accumulation of liver triglycerides. Carbon tetrachloride, ethionine, phosphorus, and cerium* all appear to affect the liver in such

* Dr. M. C. Schotz (private communication), in independent experiments, did not observe a defect in post-Triton hypertriglyceridemia in cerium-poisoned rats with fatty livers. The classification of cerium fatty liver with the fatty liver of carbon tetrachloride, ethionine and phosphorus poisoning is, therefore, open to question until the present differences have been resolved.

a way that the initial conversion of plasma NEFA to liver triglycerides is relatively unaffected, whereas one or more of the subsequent steps in the conversion of liver triglycerides to plasma lipoprotein is inhibited or destroyed. The work presented in this communication thus places the fatty liver of carbon tetrachloride, ethionine, phosphorus, and cerium poisoning on a common pathophysiologic foundation. Further study of the hepatic triglyceride secretory mechanism should reveal the detailed mechanism of action of these hepatotoxic agents. In this connection it is interesting to note that a block in protein synthesis in ethionine [31] and in carbon tetrachloride [32] fatty livers has been reported. In carbon tetrachloride fatty livers [33] a block in synthesis of phospholipids has also been observed. Robinson and Harris [34] have recently reported a defect in the synthesis of plasma lipoproteins by fatty livers due to ethionine intoxication.

The situation regarding the choline deficiency fatty liver remains obscure. It seems reasonable to expect that choline deficiency might result in lack of supply of some critical phospholipid necessary either for formation or secretion of liver triglycerides as plasma lipoprotein. Indeed, it has been shown by Olson [35] that the low-density β-lipoprotein component of rat plasma was virtually abolished in rats after 14 days on choline-deficient diets, suggesting that failure of the liver to form and secrete this plasma constituent might be the basis for hepatic triglyceride accumulation in choline deficiency. Yet in the experiments reported here, which are preliminary only in the sense that the numbers of animals used were not large, choline-deficient rats with fatty livers produced a marked post-Triton hypertriglyceridemia. Although these experiments suggest the possibility that the increased liver fat of choline deficiency is not due to a breakdown of the hepatic triglyceride secretory mechanism, we do not regard these experiments as sufficient to establish this important point. It is possible, for example, that the lipid of adipose tissue in choline-deficient rats may be accessible to the solubilizing action of Triton. A further possibility is that the hepatic mechanism which converts plasma NEFA to plasma triglycerides may be intact in choline-deficient rats, but that choline deficiency affects the liver in such a way that conversion of newly synthesized hepatic NEFA to triglycerides may be unaffected whereas subsequent removal of these triglycerides is slowed down or prevented.

The studies reported here have drawn attention to a specific hepatic lesion—viz., loss of function of a hepatic triglyceride secretory mechanism, as a fundamental component in the etiology of fatty liver disease. According to our view, pathologic accumulation of fat in the liver is fundamentally a question of malfunction in intracellular transport and se-

cretion of fat by the liver. Calvert and Brody[36] and Brodie and colleagues,[37,38] in considering certain hormonal and nervous influences, have hypothesized that pathologic accumulation of lipid in the liver is primarily a question of oversupply of plasma NEFA by adipose tissue, thus emphasizing extrahepatic factors. However, an increased rate of release of NEFA from adipose tissue does not appear to be necessary to account for observed rates of increase of liver triglycerides. For example, in carbon tetrachloride-poisoned rats deposition of triglycerides in the liver occurs at a rate of approximately 3 mg. of triglycerides per gm. of liver per hour.[1] This rate is faster than in any other fatty liver disease. From data given by Laurell[28] for normal rats, it can be calculated that about 14 mg. of tripalmitin per gm. of liver per hour would accumulate if all of the plasma NEFA turnover were trapped in the liver. Thus, less than ¼ of the normal turnover of the plasma NEFA pool need be trapped to account for the fastest known rate of pathologic hepatic lipid accumulation. Frank, Horning, and Maling[39] have recently claimed that palmitate-1-C^{14} injected intravenously into carbon tetrachloride-poisoned rats entered the liver triglyceride fraction but appeared in only trace amounts in the plasma triglyceride pool. If the pathologic accumulation of plasma NEFA in the liver triglyceride pool were merely a question of oversupply of NEFA to the liver, without a defect in the capacity of the liver to deliver triglycerides to the plasma, one would not expect the plasma triglycerides to be labeled in only trace amounts. However, the result of Frank and co-workers[39] is exactly what would be predicted if the accumulation of liver triglycerides depended on failure of a hepatic triglyceride secretory mechanism. Furthermore, the claim that plasma NEFA content rises in acute ethanol intoxication, which is an important point in the oversupply hypothesis, has not been confirmed by Elko, Wooles and di Luzio.[40] Although the work of Calvert and Brody[36] and Brodie and co-workers[37,38] has revealed hormonal and central nervous system involvement in the over-all response of rats to hepatotoxins, it appears to us that the oversupply hypothesis of these workers in its present form fails to take sufficiently into account the specific nature in which the normal handling of lipids by the liver may be pathologically disturbed.

Summary

When the nonionic detergent Triton is injected intravenously into rats, a marked hypertriglyceridemia develops. This hypertriglyceridemia is due to failure of plasma triglycerides to leave the plasma compartment of the Tritonized animal. It is known that the liver is the source of the plasma triglycerides which accumulate following Triton administra-

tion to the normal rat. In rats with experimental fatty liver due to carbon tetrachloride, ethionine, phosphorus or cerium poisoning, intravenous administration of Triton was followed by a markedly reduced triglyceridemia. Triton did not affect the content of triglycerides in the liver. It is concluded from these studies that a hepatic triglyceride secretory mechanism, which normally functions to produce plasma triglycerides, is inhibited or destroyed in these instances of experimental liver injury. The nature of the pathologic lesion appeared to be such that the entrance of plasma nonesterified fatty acids into the liver and their synthesis to triglycerides were relatively unaffected, whereas secretion of hepatic triglycerides to plasma was interrupted.

In rats with fatty livers due to choline deficiency, administration of Triton was followed by a fully developed hypertriglyceridemia. This observation suggested that the hepatic triglyceride secretory mechanism was not affected in choline-deficient rats.

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