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HEPATIC LIPID METABOLISM IN CARBON TETRACHLORIDE POISONING

INCORPORATION OF PALMITATE-1-^{14}C INTO LIPIDS OF THE LIVER AND OF THE d < 1·020 SERUM LIPOPROTEIN*

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Abstract—The incorporation of palmitate-1-^{14}C and the release of lipids into the density (d) < 1·020 lipoprotein was estimated in the isolated perfused rat liver preparation obtained from normal rats and from animals poisoned with CCl_4. Livers from normal rats released triglyceride, phospholipid, and cholesterol into the d < 1·020 lipoprotein of the perfusate, and this net release was inhibited after CCl_4 poisoning of the animal from which the liver was obtained. CCl_4 poisoning depressed the incorporation of palmitate-1-^{14}C into triglyceride, phospholipid, and cholesterol of the d < 1·020 lipoprotein. Although no statistically significant chemical change in the hepatic concentration of triglyceride, phospholipid, or cholesterol could be detected under these experimental conditions during the course of the perfusion of livers from either normal or CCl_4-poisoned rats, palmitate-1-^{14}C incorporation into liver triglyceride was increased, whereas incorporation into liver phospholipid was depressed by CCl_4 intoxication. The decreased incorporation of palmitate into the d < 1·020 lipoprotein triglyceride and the increased incorporation into liver triglyceride may reflect primarily decreased release; the decreased incorporation of palmitate into the d < 1·020 lipoprotein phospholipid coupled with a decreased incorporation into liver phospholipid may reflect not only decreased phospholipid release but depressed hepatic phospholipid biosynthesis. In confirmation of earlier work, triglyceride, phospholipid, and cholesterol were released into the d < 1·020 lipoprotein in discrete molar ratios to each other, suggesting that the very low density lipoprotein is released as a unit for the transport of triglyceride. If this lipoprotein is released as a unit, the theorem can be postulated that interference with the availability or biosynthesis of any component of the lipoprotein—the phospholipid, cholesterol, or protein—may decrease triglyceride release by the liver and be one mechanism, though certainly not an exclusive one, for the induction of the fatty liver.

IT HAS been concluded by several different investigators that the primary biochemical lesion which appears to be responsible for the induction of fatty liver by CCl_4 is the inhibition of hepatic release of triglyceride.[1–5] We reported previously that the net release of triglyceride by the isolated, perfused rat liver was diminished considerably subsequent to the administration of CCl_4 either to the animals from which the livers were removed[2] or to the medium perfusing livers obtained from normal rats.[6] During

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the perfusion of livers from normal animals not receiving CCl_4, the increase in concentration of triglyceride in the very low density lipoproteins (d* < 1·006) of the medium was accompanied by a proportional increase in the concentration of phospholipid and cholesterol in this lipoprotein fraction.[7] Furthermore, the inhibition of net triglyceride release into the d < 1·006 lipoprotein fraction induced by the administration of CCl_4 was accompanied by a simultaneous reduction in the hepatic output of phospholipid and cholesterol. It has been suggested that the triglyceride, phospholipid, and cholesterol of the d < 1·006 lipoprotein were secreted by the liver in constant proportions in order to maintain the physical stability of a lipoprotein carrier necessary for the transport of triglyceride to other tissues. The experiments reported here were carried out to further test this hypothesis. For this purpose, livers from normal animals and from animals pretreated with CCl_4 were perfused *in vitro* with a medium containing palmitate-1-^{14}C. The incorporation of radioactivity into the lipids of the liver, and into the very low density lipoprotein fraction (d < 1·020) isolated from the perfusate was measured. The changes in concentrations of the lipid components of these fractions were also determined. The results of these experiments have permitted us to extend a previous report[7] concerning the proportionality of triglyceride, phospholipid, and cholesterol release by the liver into the very low density lipoproteins. Furthermore, we have observed that the fatty liver resulting from CCl_4 poisoning is associated with a decreased synthesis of hepatic phospholipid.

METHODS

Male Sprague-Dawley rats weighing 250–350 g (obtained from Holtzman and Co., Madison, Wis.) were maintained on water and standard laboratory chow *ad libitum*. The animals from which the normal livers were removed received no further treatment. Carbon tetrachloride was administered to other animals by gastric intubation, 0·25 ml CCl_4/100 g body weight, 3·5 hr prior to surgical removal of the liver from the rat. Blood for all the perfusions was obtained from the abdominal aorta of normal rats anesthetized with ether. The perfusion procedures[8] and apparatus[9] have been described previously. After hepatectomy and insertion of the liver into the perfusion system, the liver was equilibrated for approximately 20 min with a medium which contained 43 ml defibrinated rat blood, 57 ml Krebs-Henseleit bicarbonate buffer (pH 7·4[10]), and 500 units heparin. After the equilibration period, 30 ml of a palmitate–serum complex[2] containing 50 mg palmitic acid, 5·0 μc palmitic acid-1-^{14}C (30·8 mc/m-mole), 20 ml rat serum, and 10 ml 0·9% NaCl, was added to the reservoir. After an additional period of 5 min was allowed for mixing the serum–palmitate complex, 40 ml perfusate was withdrawn as the zero-time sample. After 3-hr perfusion, a terminal aliquot of perfusate was obtained. The perfusate samples were centrifuged lightly to sediment the erythrocytes. The supernatant serum was adjusted to a density of 1·020 and the lipoproteins with d < 1·020 were isolated by the ultracentrifugal method of Havel *et al.*[11] The d < 1·020 LP fraction was lyophilized and the lipids extracted with $CHCl_3$:CH_3OH (2:1, v/v) as described previously.[7]

Samples of liver were removed for chemical and isotopic measurements immediately after the appropriate aliquots of perfusate had been obtained. The zero-time liver sample was the caudate lobe. The circulation to the caudate lobe was interrupted with

* Abbreviations used: density, d; triglyceride, TG; phospholipid, PL; cholesterol, C; cholesteryl esters, CE; diglyceride, DG; lipoprotein, LP.

umbilical tape and the lobe was excised, blotted to remove as much perfusate as possible, and then weighed. At the end of the perfusion period the remaining liver tissue was removed from the apparatus, perfused with ice-cold 0·9% NaCl, cleansed of nonhepatic tissues, blotted, and weighed. The liver samples were homogenized in 95% ethanol and brought to the boil in a water bath, filtered, and the residues extracted with ethyl ether for 8 hr in a Soxhlet apparatus. The ethanol and ether extracts were then combined, evaporated to dryness *in vacuo*, extracted with petroleum ether (b.p. 30°–60°), and dried with anhydrous Na_2SO_4.

Aliquots of the lipid extracts obtained from the serum, the d < 1·020 lipoprotein, and the liver were fractionated into pure lipid classes by means of column and thin-layer silicic acid chromatography.[7] For chemical determination of the lipid classes, triglyceride and diglyceride were analyzed by the method of Van Handel and Zilversmit.[12] Cholesteryl esters, after saponification by the method of Abell *et al.*,[13] and cholesterol, were determined by the procedure of Zak *et al.*[14] Lipid-soluble phosphorus was estimated by the method of King.[15] The incorporation of labeled palmitic acid into these lipids was measured in a liquid scintillation counter, Tracerlab model LSC-10B.[8]

RESULTS

The incorporation of palmitic acid-1-^{14}C into total liver lipids is shown in Table 1. The palmitic acid was incorporated to an equal extent into the liver lipids of both control and experimental groups. In CCl_4 poisoning, however, the incorporation of

TABLE 1. INCORPORATION OF PALMITIC ACID-1-^{14}C INTO LIPIDS OF LIVER AND PERFUSATE*

	Normal	CCl_4	P†
I. *Liver*	(3)	(4)	
Administered dis/min incorporated into total liver lipids/g liver, wet wt. (%)	5·5 ± 0·2	5·7 ± 0·2	<0·50
Administered dis/min incorporated into lipids of the F_1 fraction/g ilver, wet wt. (%)‡	2·9 ± 0·3	4·1 ± 0·2	<0·05
Dis/min in F_1 fraction present as triglycerides (%)	86·3 ± 4·2	87·8 ± 3·1	<0·80
Administered dis/min incorporated into lipids of the F_2 fraction/g liver, wet wt. (%) §¶	2·3 ± 0·2	1·2 ± 0·1	<0·001
II. *Perfusate*	(5)	(6)	
Administered dis/min incorporated into total TG/g liver, wet wt. (%)	0·71 ± 0·07	0·08 ± 0·01	<0·001
Administered dis/min incorporated into total PL/g liver, wet wt.¶	0·12 ± 0·05	0·04 ± 0·01	<0·20

* All values in all tables are means ± s.e.; the numbers of observations are shown in parentheses The livers were perfused for 3 hr with 3·47 μc palmitate-1-^{14}C (remaining after removal of initial perfusate sample).

† P indicates significance of difference between normal and CCl_4-treated groups. Probability (P) values for all tables are taken from a two-tailed table of Student's values for t.

‡ F_1 fraction includes all lipids (excluding phospholipids) present in $CHCl_3$ eluates of 3·0 g silicic acid columns.[7]

§ F_2 fraction includes phospholipids present in CH_3OH eluates of 3·0 g silicic acid columns.[7]

¶ PL were purified by thin-layer silicic acid chromatography, with the solvent mixture petroleum ether:ethyl ether:glacial acetic acid, 84:15:1, v/v.[7] The PL were recovered from thin-layer plates as described by Biezenski.[37] By this method we were able to recover only 81·9% of standard PL mixtures (N. B. Fizette and M. Heimberg, unpublished data).

TABLE 2. INCORPORATION OF PALMITATE-1-^{14}C INTO LIPIDS OF THE PERFUSED LIVER*

	TG		DG		C		CE		PL	
	TI†	SA‡	TI	SA	TI	SA	TI	SA	TI	SA
I. Normal (4)	173,253 ± 5900	19,524 ± 1660	4382§ ± 142	7504 ± 611	2603 ± 271	3596 ± 1436	2110 ± 430	2892 ± 843	130,444 ± 18,720	5057 ± 281
II. CCl_4 (5)	259,951 ± 21,300	14,839 ± 1819	6142 ± 1285	10,763 ± 2745	5413 ± 1685	5061 ± 1770	2140 ± 1458	2980 ± 1960	74,826 ± 6640	3502 ± 324
P¶	<0·01	<0·10	<0·40	<0·50	<0·20	<0·60	<0·99	<0·98	<0·02	<0·02

* Incorporation into liver lipids after 3-hr perfusion.
† Total incorporation into liver lipids; dis/min/g liver, wet wt.
‡ Specific activity; dis/min/μmole lipid.
§ Three observations only.
¶ Significance of difference between normal and CCl_4-treated groups.

Liver weights, determined at the end of the perfusion period, were 10·25 ± 0·79 and 13·20 ± 0·49 (g ± S.E., wet wt., for normal and CCl_4-treated groups respectively; $P < 0·02$). Fat-free, dry liver weights of these comparable groups were 2·34 ± 0·12 and 2·23 ± 0·17 g respectively. Bile output and perfusate flow rates, as reported previously,[6] were depressed by treatment with CCl_4 of the animal from which the liver was obtained. The measurements for bile flow and flow rate listed below are those taken at zero time and at hourly intervals thereafter.

	Hour			
	0	1	2	3
I. *Bile flow* (cumulative, ml/g liver, wet wt.)				
A. Normal	0*	0·049 ± 0·008	0·095 ± 0·008	0·124 ± 0·010
B. CCl_4	0*	0·028 ± 0·007	0·050 ± 0·012	0·070 ± 0·013
II. *Flow rate* (ml/min/g liver wet wt.)				
A. Normal	1·7 ± 0·2	2·6 ± 0·4	2·3 ± 0·4	1·7 ± 0·6
B. CCl_4	1·4 ± 0·1	1·8 ± 0·2	1·6 ± 0·2	1·0 ± 0·3

* Bile flow at this time, usually about 0·015 ml/g liver, was considered to be zero, and was subtracted from readings taken at other time periods.

palmitate-1-^{14}C into liver TG was increased, whereas incorporation into liver PL was diminished. The incorporation of palmitate-1-^{14}C into either PL or TG of the perfusate was depressed uniformly after CCl_4 intoxication. The incorporation of palmitic acid-1-^{14}C into specific lipids of perfused livers from normal and CCl_4-poisoned animals is shown in Table 2. The total incorporation into hepatic TG was increased as a consequence of CCl_4 poisoning of the rats from which the livers were obtained; the TG-specific activity was of the same order of magnitude in both groups. In contrast to the TG, both the total incorporation of radioactive palmitate into hepatic phospholipids and the specific activity were depressed by CCl_4 poisoning. The incorporation of labeled palmitate into hepatic diglyceride, cholesterol, and cholesteryl esters was almost negligible in comparison with triglyceride and phospholipid, and, moreover, did not appear to be influenced by CCl_4 intoxication.

TABLE 3. LIPID CONCENTRATIONS IN LIVER DURING COURSE OF PERFUSION*

	TG	DG	C	CE	PL
I. Normal (4)					
Time (hr)					
0	9·28 ± 0·28	1·19†	3·21	1·27	21·90 ± 4·63
3	9·12 ± 1·28	0·57†	2·99	0·78	25·49 ± 2·38
Δ‡	−0·16 ± 1·37	−0·62 ± 0·28	−0·22 ± 0·66	−0·49 ± 0·13	3·59 ± 2·38
P§	<0·95	<0·20	<0·80	<0·05	<0·30
II. CCl_4 (5)					
0	13·73 ± 1·02	1·87	2·17	1·44	30·51 ± 4·00
3	19·22 ± 3·73	0·68	2·16	0·60	21·50 ± 1·30
Δ	+5·49 ± 2·95	−1·19 ± 0·12	−0·01 ± 0·44	−0·84 ± 0·19	−9·01 ± 5·36
P§	<0·20	<0·001	<0·99	<0·02	<0·20
P¶	<0·01				
P**	<0·20	<0·10	<0·80	<0·20	<0·10

* Lipid concentrations are given as μmoles lipid/g liver, wet wt.

† Three observations only.

‡ Change which occurred during 3-hr perfusion.

§ Significance of change in concentration during period 0–3 hr for livers from normal and CCl_4-poisoned rats respectively.

¶ Significance of the differences of hepatic TG concentration present at zero time in livers from normal and CCl_4-poisoned rats respectively.

** Significance of the differences of change in lipid concentration between livers from normal and CCl_4-poisoned animals.

The changes in the concentration of the various classes of hepatic lipids which occurred during the course of the perfusion of livers from normal and CCl_4-poisoned animals are indicated in Table 3. We failed to observe any statistically significant change in hepatic triglyceride or phospholipid concentration during the course of the perfusion regardless of whether the livers were obtained from normal or CCl_4-poisoned animals. The concentration of hepatic cholesterol remained constant during all the perfusions; the concentration of cholesteryl esters, however, was diminished significantly during perfusion of livers from both normal and CCl_4-poisoned animals. There appeared to be a decrease in the concentration of hepatic diglyceride during the course of the perfusion, although this change was not statistically significant with the small number of observations in the control group.

TABLE 4. LIPID CHANGES IN THE d < 1·020 LIPOPROTEIN DURING THE COURSE OF PERFUSION

	TG		DG		C		CE		PL	
	Content*	Release†	Content	Release	Content	Release	Content	Release	Content	Release
I. Normal (4)										
Time (hr)										
0	6·55		0·62		1·20		1·22		2·73	
3	12·24		0·73		2·17		1·12		4·73	
Δ‡	5·69	0·446	0·12	0·008	0·97	0·077	−0·10	−0·010	2·00	0·158
	± 1·10	± 0·095	± 0·06	± 0·007	± 0·24	± 0·016	± 0·13	± 0·010	± 0·45	± 0·034
P§	<0·02	<0·02	<0·20	<0·40	<0·05	<0·02	<0·50	<0·40	<0·02	<0·02
II. CCl_4 (6)										
0	5·41		0·50		0·92		1·08		1·84	
3	2·08		0·31		0·70		0·72		1·05	
Δ	−3·33	−0·226	−0·19	−0·017	−0·22	−0·014	−0·36	−0·024	−0·79	−0·053
	± 1·25	± 0·084	± 0·13	± 0·010	± 0·12	± 0·008	± 0·16	± 0·010	± 0·34	± 0·022
P§	<0·05	<0·05	<0·30	<0·20	<0·20	<0·20	<0·10	<0·10	<0·10	<0·10
P¶		<0·001		<0·20		<0·001		<0·40		<0·001

* Content of lipid is expressed as μmoles lipid in the d < 1·020 lipoprotein/100 ml perfusate.

† Release of lipid is expressed as the change of μmoles lipid in the d < 1·020 lipoprotein of the perfusate/g liver, wet wt., during the 3-hr perfusion period. Negative numbers imply net uptake of lipid.

‡ Change in content during the perfusion period.

§ Significance of the changes in content and net release of lipid classes by livers from normal and CCl_4-poisoned rats respectively.

¶ Significance of the differences in release of lipid classes between livers from normal and CCl_4-poisoned rats.

The release of lipids by the liver into the $d < 1{\cdot}020$ lipoprotein of the perfusate is shown in Table 4. The data reported here confirm and extend results reported previously.[7] It was observed that livers from normal animals released statistically significant increments of triglyceride, phospholipid, and cholesterol into the $d < 1{\cdot}020$ lipoprotein; there was no change in the concentration of diglyceride or cholesteryl esters in the $d < 1{\cdot}020$ lipoprotein. Livers from animals poisoned with CCl_4 failed to release net quantities of triglyceride, phospholipid, and cholesterol into the $d < 1{\cdot}020$ lipoprotein; indeed, a significant decrease of TG concentration in this lipoprotein was observed. The incorporation of palmitate-1-^{14}C into the lipids of the $d < 1{\cdot}020$ lipoprotein is illustrated in Table 5. The livers from animals which had been treated with CCl_4, in comparison to the normal, incorporated less radioactivity from palmitate-1-^{14}C into the triglyceride, diglyceride, phospholipid, and cholesterol of the $d < 1{\cdot}020$ lipoprotein. Furthermore, the specific radioactivity of the triglyceride and phospholipid in the lipoprotein was reduced significantly. The reduction of incorporation of palmitate-1-^{14}C into the triglyceride and phospholipid of the $d < 1{\cdot}020$ lipoprotein in CCl_4 intoxication is a reflection of the decrease in net output of these lipid classes; in the case of the phospholipid, however, the low levels of radioactivity also result from depressed hepatic phospholipid biosynthesis.

DISCUSSION

Although triglyceride accumulates in the livers of intact animals poisoned with CCl_4, we were not able to demonstrate a statistically significant accumulation of chemically measurable hepatic triglyceride during the perfusion of livers from either normal or CCl_4-poisoned rats. These results may be attributed, in part, to the following: (1) the theoretical limitation for triglyceride formation from palmitate under the conditions of our experiments, (2) the variations in the concentration of triglyceride between the caudate lobes and the larger liver lobes, and (3) the amount of nonesterified fatty acids (NEFA) presented to the liver in these experiments. From the data presented in Table 1, it can be calculated that 25·6 per cent [e.g. (2·9) (0·863) (10·25)] of the administered palmitate-1-^{14}C could be recovered in hepatic triglyceride per average normal liver. If we assume that the unlabeled fatty acid was in equilibrium with the labeled palmitate and that all triglyceride synthesized came from palmitate, only 135 μmoles of added palmitic acid were available to the liver during the 3-hr perfusion period from which, at most, 1·12 μmoles TG/g liver could arise [(135) (0·33) (0·256) $\div$ 10·25]. This would represent an increase in hepatic triglyceride concentration of 12·10% above the initial level of 9·28 μmoles TG/g liver. We can conclude from similar calculations for livers from CCl_4-poisoned animals that approximately 1·62 μmoles TG/g liver could accumulate in the liver during perfusion, representing an increase of 11·8 per cent over the starting level. If one includes an additional 0·77 μmole TG/g which was removed from the whole perfusate by the livers from CCl_4-poisoned animals, this still represents a maximal increase of only 17·4 per cent above the 13·73 μmoles TG/g liver present at the start of the perfusion.

Furthermore, in this series of observations, the variation in triglyceride concentration among caudate lobes (from which the initial triglyceride levels were obtained) and the cystic or left lobes of the liver was larger than the theoretical increase one could expect as a result of conversion of the palmitic acid to triglyceride (Table 6).

TABLE 5. INCORPORATION OF PALMITATE-1-^{14}C INTO LIPIDS OF d < 1·020 LIPOPROTEIN*

	TG		DG		C		CE		PL	
	TI†	SA‡	TI	SA	TI	SA	TI	SA	TI	SA
I. Normal (4)	28,428 ± 4570	29,550 ± 3230	597 ± 165	10,075 ± 1805	220 ± 44	1144 ± 213	365 ± 159	4300 ± 1880	3300 ± 1355	7980 ± 2452
II. CCl_4 (6)	2523 ± 683	16,335 ± 1012	136 ± 17	14,215 ± 6450	97 ± 12	2058 ± 246	132 ± 12	3200 ± 822	196 ± 33	2953 ± 614
P§	<0·001	<0·001	<0·01	<0·70	<0·02	<0·05	<0·20	<0·60	<0·02	<0·05

* Incorporation into d < 1·020 lipoprotein lipids of the perfusate during a 3-hr perfusion period.
† Total incorporation into d < 1·020 LP lipids; dis/min/g liver, wet wt.
‡ Specific activity; dis/min/μmole lipid.
§ Significance of differences between normal and CCl_4-treated groups.

Although one would hope to observe chemical accumulation of triglyceride in perfused livers in which release of triglyceride had been inhibited by treatment of the animal with CCl_4, this did not prove to be the case under our experimental conditions. It is reasonable to assume that a much larger supply of plasma NEFA must be made available to the poisoned liver in order to measure increases in hepatic triglycerides

TABLE 6. DISTRIBUTION OF TRIGLYCERIDE AND PHOSPHOLIPID IN VARIOUS LOBES OF RAT LIVER

Liver section	Concentration*			
	TG		PL	
	Normal (5)	CCl_4 (3)	Normal (5)	CCl_4 (3)
Caudate lobe	10·43 ± 2·87	22·98 ± 6·97	42·04 ± 4·77	49·43 ± 13·21
Rt. cystic lobe	7·02 ± 0·56	15·16 ± 3·48	43·76 ± 4·39	40·41 ± 4·87
Left lobe	6·71 ± 0·52	15·79 ± 2·48	48·12 ± 4·31	34·03†

* Liver concentration of either TG or PL is expressed as μmoles lipid/g liver, wet wt. These livers were not perfused (as described in text) but were taken directly from the animal.

† Two observations only.

which are statistically significant. In contrast to the results of the chemical measurements, it is clear that the livers from CCl_4-poisoned animals accumulated radioactivity from palmitate-1-^{14}C in the hepatic triglyceride to a much greater extent than did livers from normal rats. The accumulation of labeled hepatic triglyceride was associated with a decreased release of triglyceride from the liver into the perfusate, measured by either chemical or isotopic means.

The specific radioactivity of the TG of the $d < 1{\cdot}020$ lipoprotein lipids appeared to be of a greater magnitude than the specific activity of the hepatic triglyceride from which it was derived. It may be inferred from these data that the newly formed triglyceride of the serum lipoproteins did not equilibrate evenly with the total TG content of the liver. This information would presume the existence of a minimum of two distinct functional hepatic triglyceride pools—e.g. a storage pool(s) and a metabolic pool(s). This postulate has been proposed by several investigators who have obtained various types of experimental evidence.[3, 5, 7, 16, 17]

We reported previously that the release of TG into the $d < 1{\cdot}006$ lipoprotein was accompanied by a proportional release of phospholipid and cholesterol, and that the release of these lipid classes into the very low density lipoprotein was inhibited by CCl_4 intoxication.[7] In the present study, all lipoproteins with a $d < 1{\cdot}020$ were collected as one fraction, since, in rat serum, the lipoproteins with d 1·006 to 1·020 appear to contain little lipid. The data obtained in the present study with all the $d < 1{\cdot}020$ lipoprotein, confirmed and extended the previous studies with the $d < 1{\cdot}006$ lipoprotein, showing the proportionality of triglyceride, phospholipid, and cholesterol release. The combined molar ratios of lipids released by livers from normal rats into the $d < 1{\cdot}020$ lipoproteins observed in these experiments (e.g. TG:PL:C = 1·000:0·354:0·172) and in those reported previously are TG:PL:C = 1·000:0·326 ± 0·024:0·172 ± 0·017. (These ratios are based on the means of μmoles

lipid released in the d < 1·020 LP/g liver ± s.e. The correlation coefficients for TG:PL and TG:C were 0·922 and 0·904 respectively, each with $P < 0{\cdot}01$. These calculations were made from the release data of Table 4, in which release of TG—e.g. 0·466 μmole/g liver—was considered to be unity. Similar ratios can not be calculated for livers from CCl_4-poisoned animals since there was no net release of TG, PL, or C into the d < 1·020 lipoprotein of the perfusate.)

If the triglyceride, phospholipid, and cholesterol of the d < 1·020 lipoprotein are secreted by the liver in fixed proportions in order to provide the necessary physical properties for the dispersion of these lipids in an aqueous environment, and if this lipoprotein fraction functions as a vehicle for TG transport,[7] then it is not unreasonable to suggest that interference with the synthesis or availability of any component of the lipoprotein unit, either lipid or protein moiety, could result in decreased capability of the liver to release TG. If the limiting factor in the secretion of the lipoprotein is neither TG nor TG precursors, then inhibition of release could be one mechanism for the induction of a fatty liver rich in triglyceride. It is clear that hepatic release of TG is not proportional in any simple fashion to its content in the liver, since TG release fails to occur in a variety of situations associated with increased hepatic triglyceride concentration. The hepatic synthesis of phospholipid has been reported in these experiments and has been observed earlier by other workers[4, 18] to be inhibited in CCl_4 intoxication. The inhibition of incorporation of palmitate-1-^{14}C into phospholipid in CCl_4 poisoning may make a larger pool of plasma NEFA available for esterification to form triglyceride. Furthermore, if phosphoplipid is required as an essential component for the release of TG from the liver in the very low density lipoprotein unit, it is conceivable that PL synthesis may be another rate-limiting factor in the normal mechanisms of hepatic TG release. The failure of hepatic TG secretion in CCl_4 poisoning may, in part, be a consequence of decreased PL synthesis.

The inhibition of TG release by livers from alloxan-diabetic animals,* or from animals treated with ethionine[19, 20] may also, in part, be a consequence of decreased PL synthesis. Although hepatic PL synthesis is depressed in choline deficiency,[21] it is not at all clear that the fatty liver induced by choline deficiency is related to an inhibition of hepatic TG release. Although fasted rats given a choline-deficient diet for 1–3 days had no apparent inhibition of hepatic TG release (as measured by lack of effect of the diet on post-Triton hyperlipemia[22] or on the incorporation of palmitate-1-^{14}C into liver and serum TG[23]), it has been shown that perfused livers obtained from rats maintained for periods of 5–30 days on choline-deficient diets exhibited either a reduced fractional turnover rate of hepatic lipid[24] or showed a marked inhibition of TG release into the perfusate.[25] Pharmacolgic agents that inhibit hepatic cholesterol biosynthesis conceivably may also reduce hepatic TG release if they restrict the formation of cholesterol which has been postulated as an essential moiety of the serum lipoprotein transport system for triglyceride. It has been reported that chronic administration of hypocholesterolemic agents such as triparanol[26] and SKF 525-A[27, 28] produced fatty infiltration of the liver. Finally, various conditions which result in a reduction of the biosynthesis of serum lipoprotein protein are inhibitory to triglyceride release from the liver, and are productive of a fatty liver. It has been known for a long time that dietary protein restriction[29] or amino acid deficiency or imbalance[29–33] will give rise to a fatty liver. Inhibition of lipoprotein

protein biosynthesis is seen in CCl_4 poisoning,[4, 34] after ethionine administration,[20] in experimental diabetes,† or after treatment of the animal with puromycin[35]—conditions which induce fatty livers characterized by increased hepatic triglyceride concentrations. It is not inconceivable that the fatty liver which has been described as a toxic manifestation of tetracycline administration in man may be a result of the inhibitory effects of this class of antibiotics on protein synthesis.[36]

* M. Heimberg, D. Van Harken, and T. O. Brown; unpublished.
† H. G. Wilcox and M. Heimberg; unpublished observations.

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