Effect of Carbon Tetrachloride on Incorporation of Linoleic-1-C^{14} Acid into Liver Lipids in Rats *in vivo*.* (31466)

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The phospholipid pattern in rat liver was shown to be altered in animals undergoing fatty degeneration of hepatic tissue after carbon tetrachloride (CCl_4) administration(1). On the other hand, it has been recognized that the uptake of various long chain fatty acids by liver phospholipids is unique, that is, certain fatty acids exhibited a preferential incorporation into specific phospholipids. In the present study, the incorporation of a metabolically active fatty acid, linoleic-1-C_{14} acid, into individual phospholipids of liver lipids was followed in rats which had been treated with CCl_4.

Methods. Eight male rats of the Sprague-Dawley strain, from 240 to 270 g in body weight, were maintained on a balanced stock ration and given water *ad libitum*. Animals serving as liver donors were fasted for 18 hours and then lightly anethesized with ether. Four rats were force-fed by gastric intubation a 1:1 carbon tetrachloride-mineral oil mixture (0.5 ml/100 g body weight). Four hours later, both the CCl_4 treated and the untreated rats were injected with 10 μC of linoleate-1-C_{14} albumin. The isotope was prepared from linoleic-1-C^{14} acid (California Corp. of Biochemical Research, Los Angeles). The tagged acid was dissolved in ethanol, neutralized with NaOH and taken to dryness. The sodium linoleate-1-C^{14} was dissolved in 10% human serum albumin so that the final solution contained 10 μC per ml. The isotope was injected into the tail vein of an untreated rat or to a CCl_4 treated rat. The animal was kept unrestrained for a 20-minute period, after which it was decapitated and the liver was removed.

Total lipid was extracted by the procedure of Folch *et al*(2) and determined by weight. Lipid soluble phosphorus was determined according to Bartlett(3), a factor of 25 was used for conversion to phospholipid. A preliminary separation into neutral lipid and phospholipid was made on a silicic acid column by eluting first with chloroform and then with absolute methanol. Phospholipids were separated on silicic acid columns(1) and were identified by thin layer chromatography(4). Fatty acids were analyzed by Gas Liquid Chromatography as previously described(1).

For radioactivity measurements 0.5 ml aliquot of each fraction, as soon as it eluted off the column, was taken, evaporated to dryness under nitrogen, taken up in 15 ml of a solution consisting of 3 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(phenyloxazolyl)-benzene per liter of toluene and counted in a Packard Tricarb, automatic Model 314 EX scintillation spectrometer.

Results and discussion. The results indicated that the untreated animals incorporated 17.99% of the injected radioactivity into the liver lipids, of which 52.8% was incorporated into the neutral lipid fraction and 47.2% into the phospholipid fraction (Table I). The CCl_4 treated animal incorporated 27.4% of the injected radioactivity into the liver lipids of which 66.7% was incorporated into neutral lipid and 33.2% into the phospholipid fraction.

These data suggest that after administration of CCl_4, labelled linoleate was actively incorporated into liver lipids. Twenty minutes after injection of the labelled acid, the CCl_4 treated animal appeared to incorporate plasma linoleate into triglycerides and phospholipids more rapidly than an untreated animal. Maling *et al*(5) reported that the incorporation of labelled palmitic acid into liver lipids of CCl_4 treated rats 10-60 minutes after injection changed considerably with the time interval of the observation. The data presented in Table I and the correlations derived from them would be representative for a 20-minute interval after injection.

The silicic acid curve for the phospholipids

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TABLE I. Effect of Carbon Tetrachloride on Incorporation of Linoleic-1-C^{14} Acid into Liver Lipids in Rats *in vivo*.

	Wt of wet liver, g	Total lipid, mg	Lipid wet wt of liver, mg/g	Phospholipid wet wt of liver, mg/g	Radioactivity as specific activity* Total lipid	Neutral fat	Phospholipid
Control rats†	9.6 ± 1.4‡	581 ± 25.3	59.4 ± 4.7	34.2 ± 2.8	6814 ± 395	8294 ± 481	5653 ± 360
CCl_4-treated	11.5 ± 2.2	1449 ±190	126 ±12.3	30.9 ± 2.6	4198 ± 276	3989 ± 278	5771 ± 295

* Expressed as disintegrations per min per mg. † Four experimental animals. ‡ Figures preceded by ± sign indicate standard deviation.

of fatty livers from the liver lipids of animals treated with CCl_4 is presented in Fig. 1. One hundred-sixty mg phospholipid (6.4 mg of phospholipid phosphorus) was applied on the column; the recovery was 94%. The phospholipid elution pattern of the liver lipids from untreated animals is given in Fig. 2. In both samples the main peaks were identified as nitrogen free, acidic phospholipids with cardiolipin (I), phosphatidyl ethanolamine (II), phosphatidyl inositol (III), phosphatidyl serine (IV), phosphatidyl choline (V), and sphingomyelin plus lysophosphatidyl choline (VI) as predominant components. The distribution of radioactive linoleic-1-C^{14} acid in the individual phospholipids in a normal liver 20 minutes after the intravenous injection is shown in Fig. 2. The curve in each figure was constructed on the basis of experimental data taken from one animal. Data obtained from the remaining rats in each group were in general agreement. The analytical data are recorded in Table II. In an untreated liver the uptake of linoleic acid was highest (67.35%) in phosphatidyl choline which also contained a high level of linoleic acid (67%). It is interesting to note that fraction I (Table III) considered to be high in "cardiolipin" or diphosphorylglyceryl glycerol which is known to contain a large percentage of linoleic acid incorporated a considerable amount of radioactivity. Phosphtidyl ethanolamine species containing lesser amounts of linoleic acid incorporated 21.72% of total radioactivity. Phosphatidyl inositol, serine and sphingomyelin together with lysophosphatidyl choline shared minor amounts of radioactive linoleic-1-C^{14} acid.

In a fatty liver the order of incorporation of tagged linoleic acid into phospholipids showed a qualitative similarity to that of a normal liver. Quantitatively, an increased uptake of phosphatidyl choline and the nitrogen-free phospholipids, and a decline in phospha-

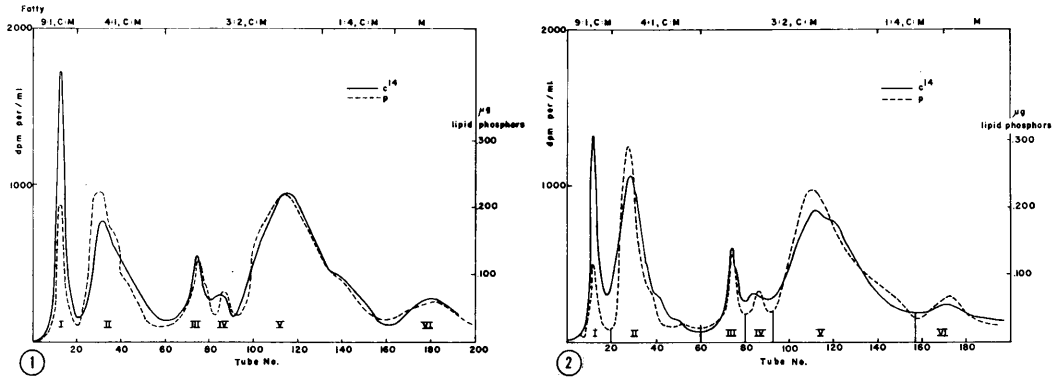

FIG. 1. Incorporation of Linoleic-1-C^{14} acid (C^{14}) into phospholipids (P) of fatty rat liver. Fraction I, nitrogen free, acidic phospholipids with cardiolipin; II, phosphatidyl ethanolamine; III, phosphatidyl inositol; IV, phosphatidyl serine; V, phosphatidyl choline; VI, sphingomyelin plus lysophosphatidyl choline. C = Chloroform. M = Methanol.

FIG. 2. Incorporation of Linoleic-1-C^{14} (C^{14}) into phospholipids (P) of normal rat liver. Fraction I, nitrogen free, acidic phospholipids with cardiolipin; II, phosphatidyl ethanolamine; III, phosphatidyl inositol; IV, phosphatidyl serine; V, phosphatidyl choline; VI, sphingomyelin plus lysophosphatidyl choline. C = Cholorform. M = Methanol.

TABLE II. Incorporation of Linoleic-1-C^{14} Acid into Individual Phospholipids of Normal and Fatty Liver 4 Hours After Carbon Tetrachloride Administration.

Fraction*	Total radioactivity†	% radioactivity	Lipid phosphate		
			μg P	% P	Specific activity‡
Normal liver					
I	68815	7.78	175	2.80	393
II	191929	21.72	1532	24.54	125
III	19707	2.23	490	7.85	39
IV	5541	.62	280	4.48	19
V	595119	67.35	3452	55.31	172
VI	2382	.26	312	4.99	7
Fatty liver					
I	89828	10.20	305	4.90	301
II	146811	16.11	1265	20.40	116
III	22790	2.64	585	9.43	40
IV	3104	.38	228	3.67	13
V	663844	71.45	3453	55.69	193
VI	2653	.32	365	5.88	6

* Experimental details in text.
† Expressed as disintegrations per min.
‡ Expressed as disintegrations per min per μg of lipid phosphate.

tidyl ethanolamine and phosphatidyl serine was noted. From the phospholipid phosphorus data recorded in Table II it is evident that phospholipid composition changed as a result of fatty degeneration.

The changes in specific activities (Table II) reflect similar results. Phosphatidyl choline had the same phosphorus value in the treated and untreated rats. An increased incorporation of linoleic acid in the CCl_4 treated animal was noted in the fatty acid composition (Table III) and could have resulted in an increased specific activity (Table II). The nitrogen-free phospholipid that comprised fraction I showed an increased incorporation of radioactivity followed by a rise in the amount of phospholipid phosphorus. As a result, the specific activity of fraction I decreased. Fraction I was a mixture of several phospholipid species and in an earlier report from this laboratory(1) it was demonstrated that phosphatidyl glycerol which is a constituent of that fraction increases considerably in a fatty liver induced by treatment with CCl_4. Since phosphatidyl glycerol is deficient in linoleic acid this could explain the observed low specific activity of that fraction. The high level of incorporated radioactivity could be due to a high turnover rate of linoleic acid in cardiolipin which is the predominant component of that fraction. In phosphatidyl ethanolamine a decrease both in trace incorporation and in the amount of radioactivity found in the CCl_4 treated liver was followed by a decrease in its specific activity although its linoleic acid content increased (Table III). To reconcile this discrepancy the assumption could be advanced that phosphatidyl ethanolamine incorporated linoleic acid from a separate pool containing significantly less labelled linoleic acid. The existence of heterogeneity in fatty acid pools associated with the uptake of fatty acids by phospholipid moieties lacks experimental support. Until this area is ex-

TABLE III. Fatty Acid Composition* of Liver Phospholipids in Control and Carbon Tetrachloride Treated Rats.†

Fatty acid	Fraction I		Fraction II		Fraction V	
	Control	CCl_4	Control	CCl_4	Control	CCl_4
C14	.3 ± .1	.2 ± .1	.2 ± .1	.5 ± .2	.2 ± .1	.2 ± .1
C16	5.4 ± .6	4.0 ± .3	17.8 ± 2.1	19.6 ± 2.9	24.5 ± 3.8	23.5 ± 3.2
C16:1‡	3.4 ± .3	2.9 ± .4	.5 ± .2	.7 ± .2	.7 ± .3	.8 ± .5
C16:2	.8 ± .2	.6 ± .1	.3 ± .1	.5 ± .2	.3 ± .1	.1 ± .0
C18	4.8 ± .7	5.4 ± 1.2	30.1 ± 4.7	34.7 ± 4.9	22.0 ± 2.9	25.8 ± 3.1
C18:1	13.1 ± .8	13.8 ± 1.1	5.7 ± 1.8	7.1 ± 1.4	9.1 ± 1.0	9.4 ± 1.1
C18:2	63.1 ± .2	65.0 ± 4.1	7.2 ± 1.6	11.3 ± 2.2	15.1 ± 1.8	21.0 ± 2.1
C18:3	—	—	.6 ± .1	.3 ± .1	.5 ± .1	.3 ± .1
C20:3	2.9 ± .8	2.4 ± .7	.4 ± .1	.3 ± .1	.5 ± .2	.6 ± .1
C20:4	5.7 ±1.4	4.2 ± .7	25.5 ± 4.1	19.7 ± 3.2	19.3 ± 2.9	14.8 ± 1.2
C20:5	—	—	1.7 ± .4	.4 ± .1	1.8 ± .4	.8 ± .3
C22:5	—	—	3.0 ± .6	.5 ± .1	2.1 ± 1.1	.4 ± .1
C22:6	—	—	7.3 ± .9	4.7 ± .5	5.1 ± .6	2.5 ± .4

* Expressed as percentage of total acids detected.
† Values are expressed as mean ± standard deviations of the mean.
‡ Number before colon represents carbon chain length, that after it represents number of the double bonds.

plored further such discrepancies remain unexplained.

Summary. Linoleic-1-C^{14} acid was rapidly incorporated into the liver lipids of both control and carbon tetrachloride treated rats. Twenty minutes after the injection, a pronounced fall in incorporation into phosphatidyl ethanolamine with a concurrent rise in incorporation into cardiolipin and phosphatidyl choline fractions was observed in the degenerating liver. The increased incorporation of radioactivity into cardiolipin and phosphatidyl choline was reflected in an increased content of linoleic acid in these phospholipids but not in phosphatidyl ethanolamine. These results suggested the existence of separate pools of linoleic acid for incorporation into individual phospholipids.

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Measurement of Bone Mass from Ultrasonic Transmission Time.* (31467)

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The major emphasis in the medical diagnostic applications of ultrasound has been on measurement of the reflections of sound from interphases within the tissue examined(1,2). Some early work was based upon absorption of sound and measurements of transit time through tissue have been made(3), but no systematic attempt to evaluate the accuracy of this last principle for measurements of body structure has previously been carried out.

The measurement we describe here is based upon transmission time and depends on the fact that velocity of sound is greater in solids than in liquids. Therefore, if a pulse of sound is passed through a limb which contains bone and soft tissues, the velocity will be greater in the bone than in the soft tissues and the time necessary for sound to traverse the limb will depend upon the distance of the sonic path in each.

Experimental. We have modified the cardiometer and sonodistometer, instruments that provide a continuous measurement of the distance between structures, such as the walls of the heart or blood vessels(4,5), to develop a system which can be used to measure the velocity of sound in any given medium. Ceramic barium titanate transducers with a natural thickness mode of vibration of 3 megacycles are fixed to each end of a rigid "U" shaped frame (Fig. 1). A 900v step of 50 nsec rise time is applied 60 times a second to one transducer to generate a series of abrupt sonic pulses. These pass through the water or other medium separating the transducers and are converted by the other transducer to a variable and often much smaller voltage, depending upon the amount of absorption or reflection of sound in the tissue examined. A linear amplifier circuit is used to amplify the signal from the receiver transducer to about 2v, within input limits between 100 μv and 10v. A voltage ramp is started at the time the transmitter transducer is pulsed and cut off by the signal from the receiver output. Because the voltage rises at a constant rate during the time between these two signals, the maximum voltage attained is directly proportional to the time between transmission and reception of the pulse. The highest volt-

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